

Figure 1

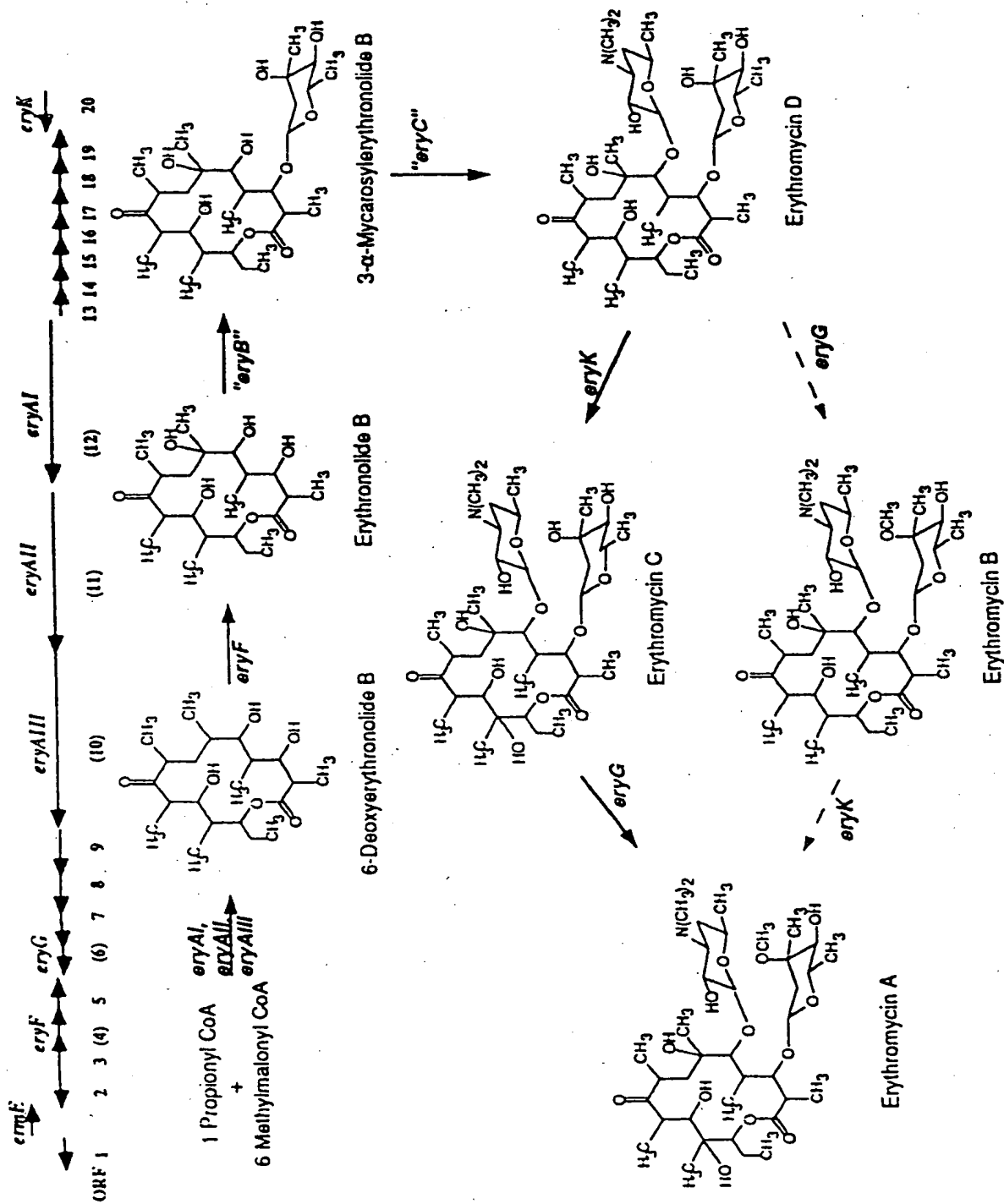


Figure 2

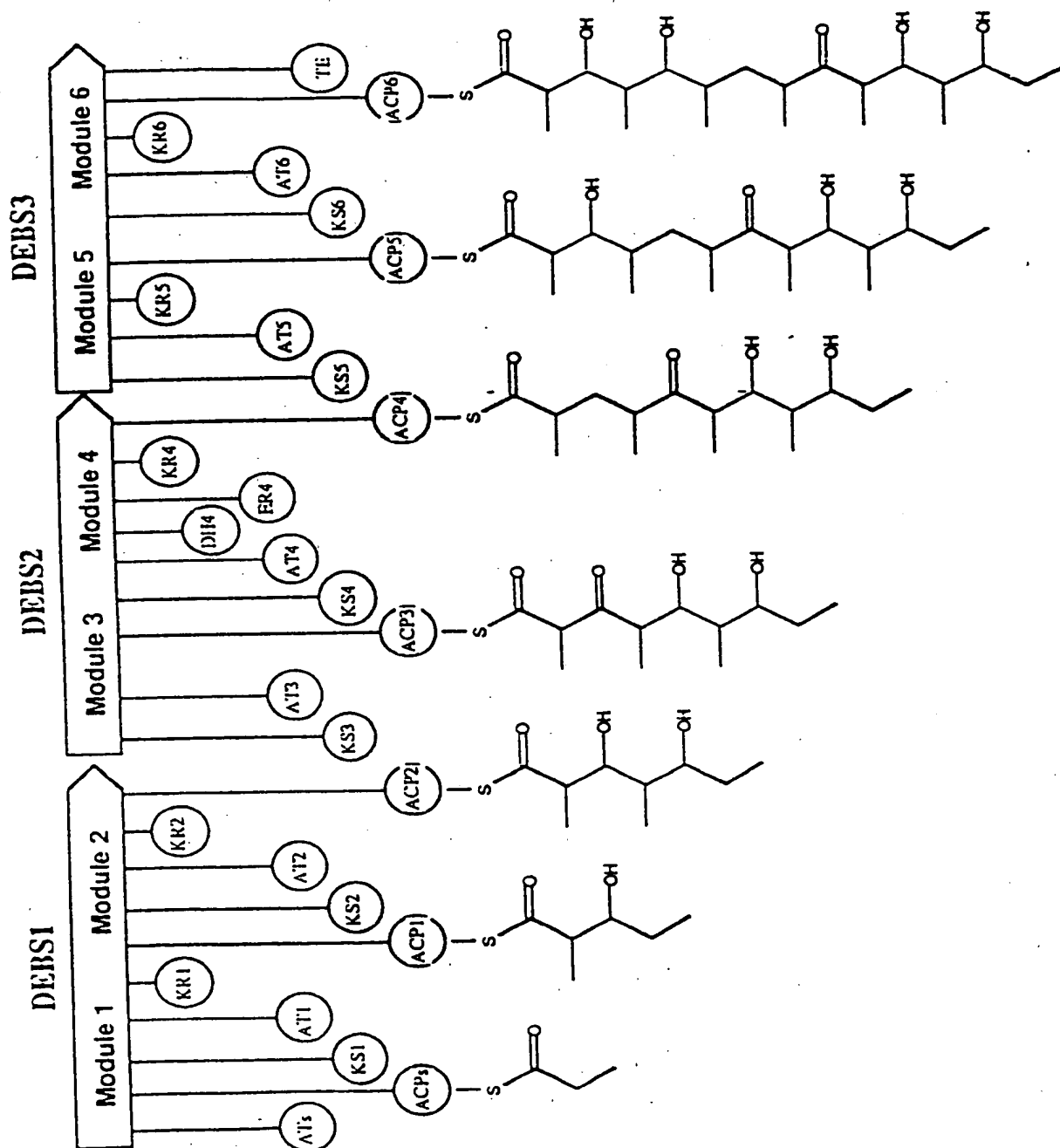
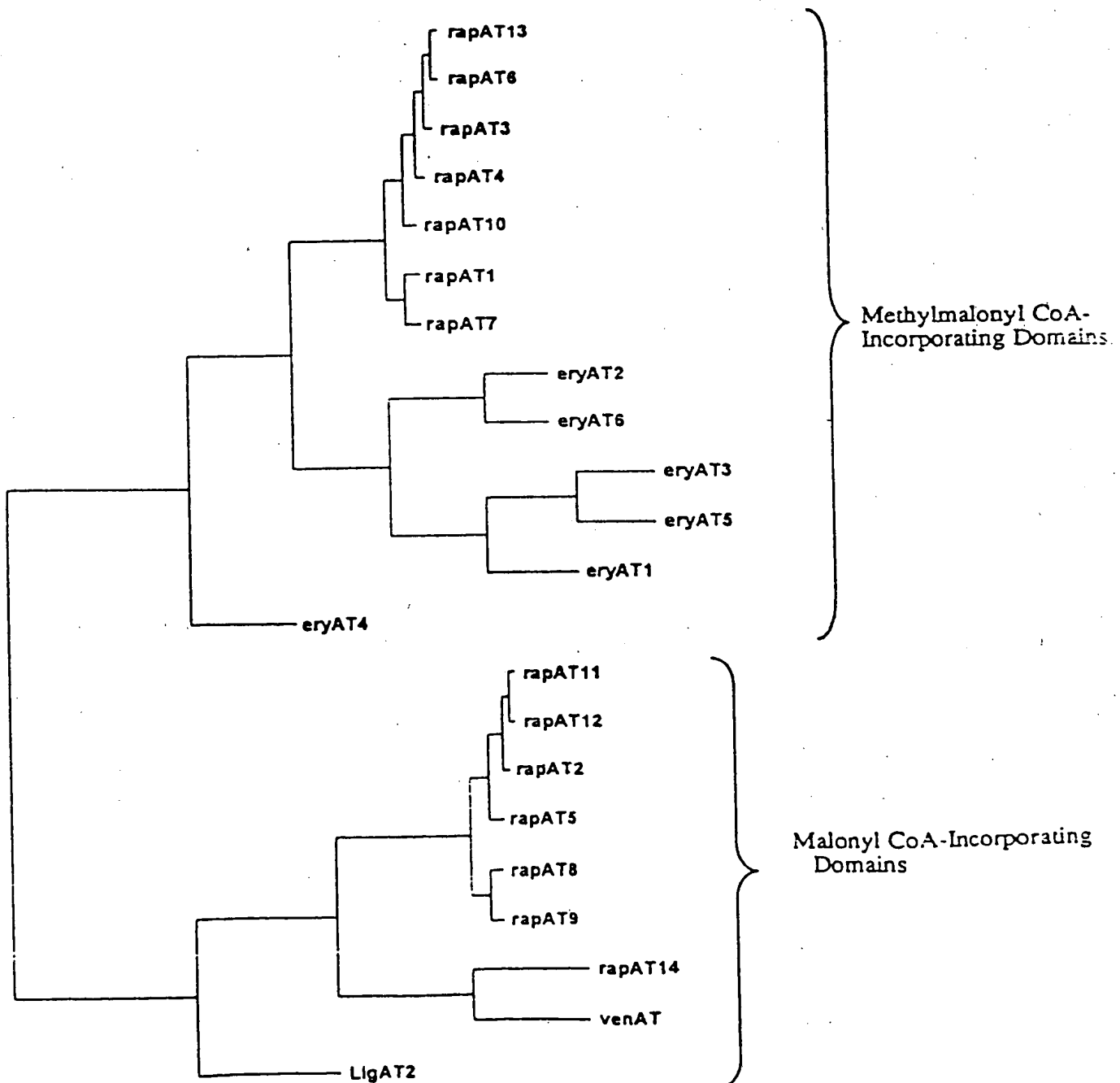
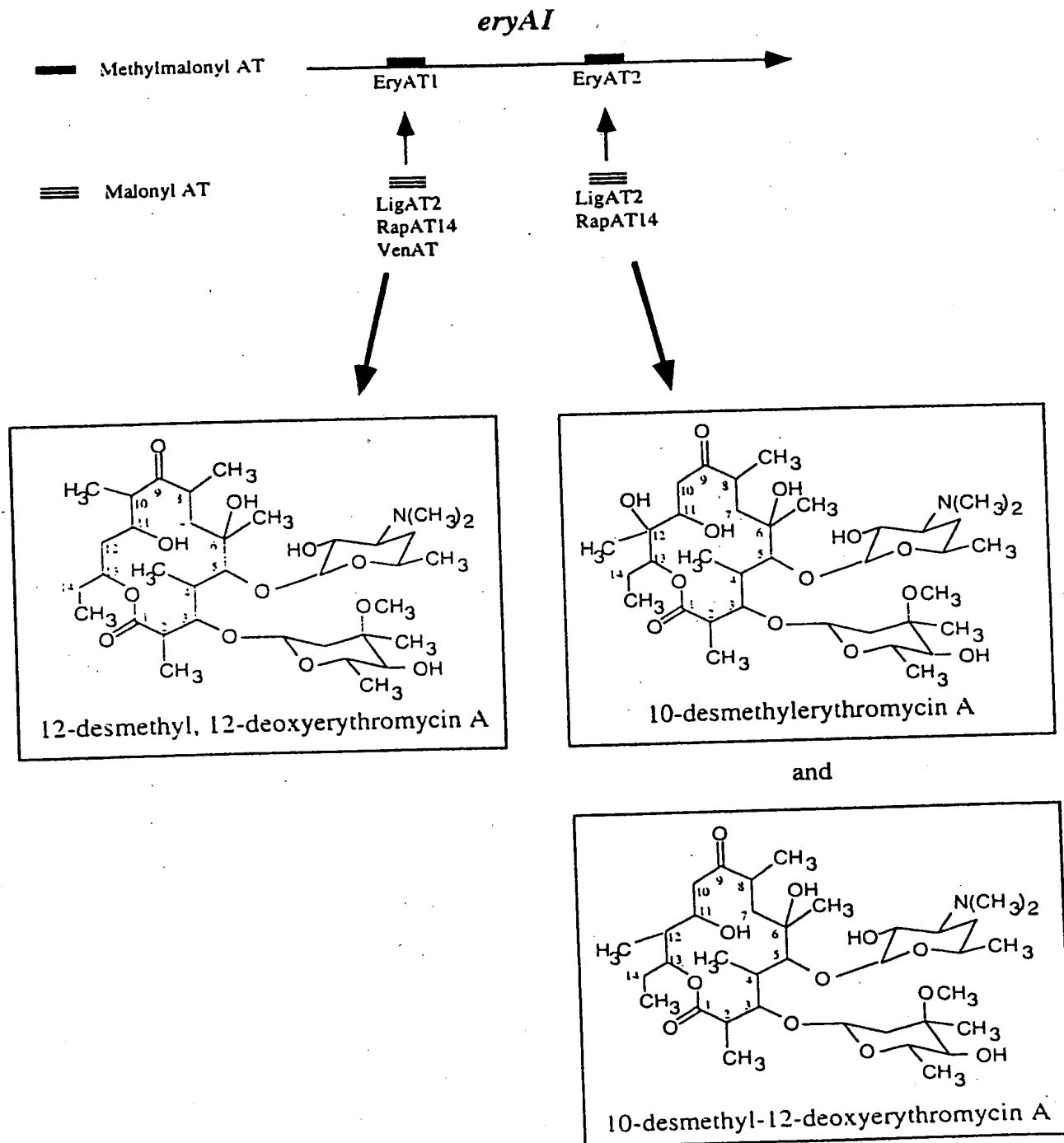


Figure 3



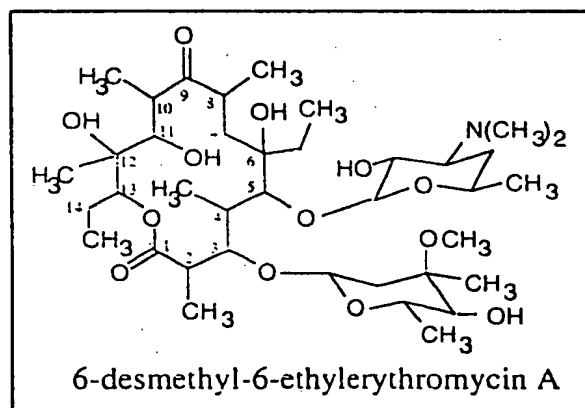
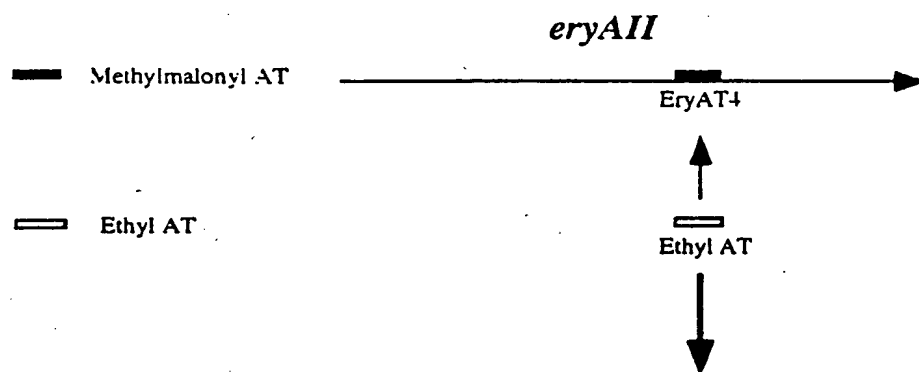
09735056-121100

Figure 4a



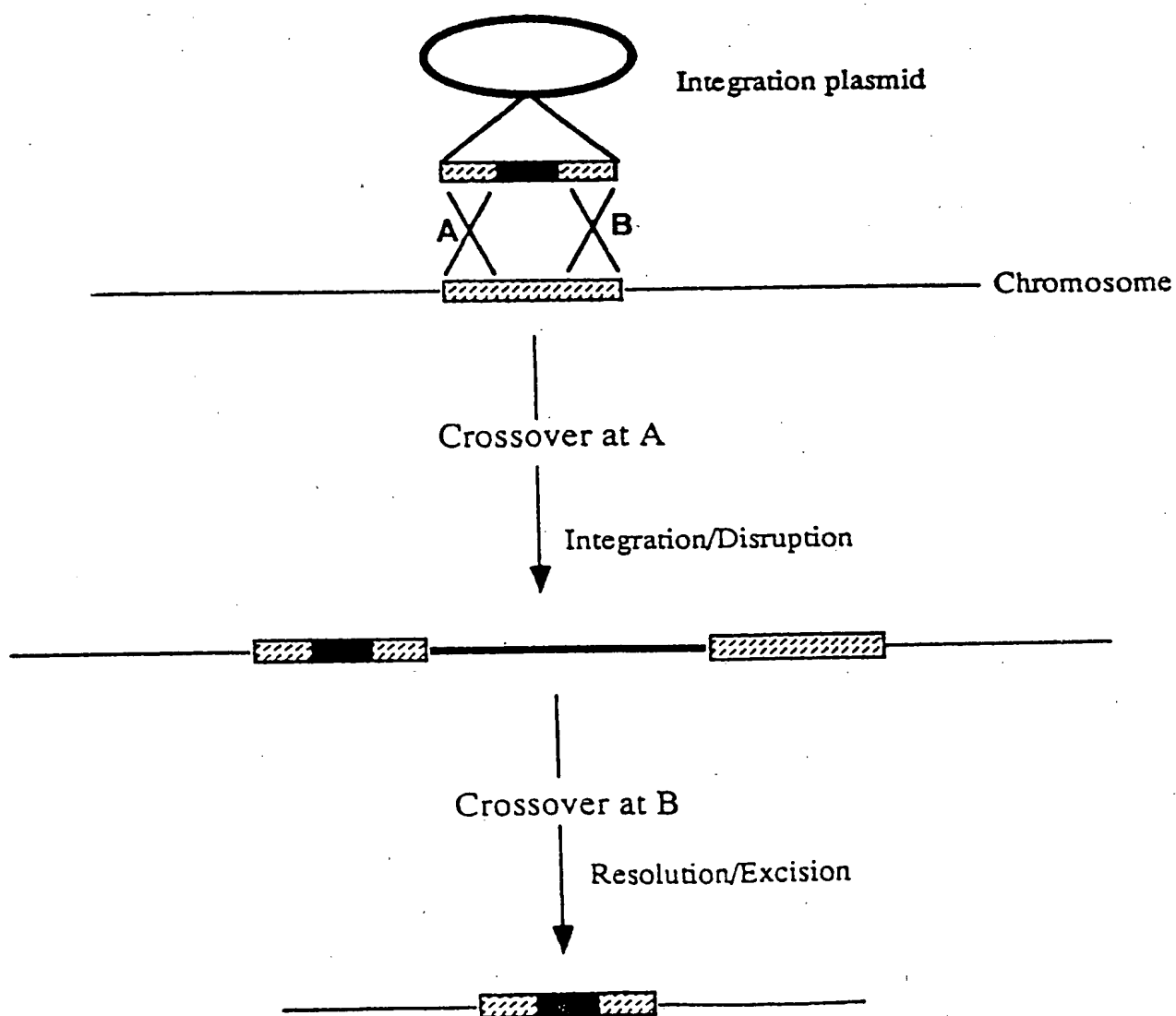
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Figure 4b



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Figure 5



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Figure 6

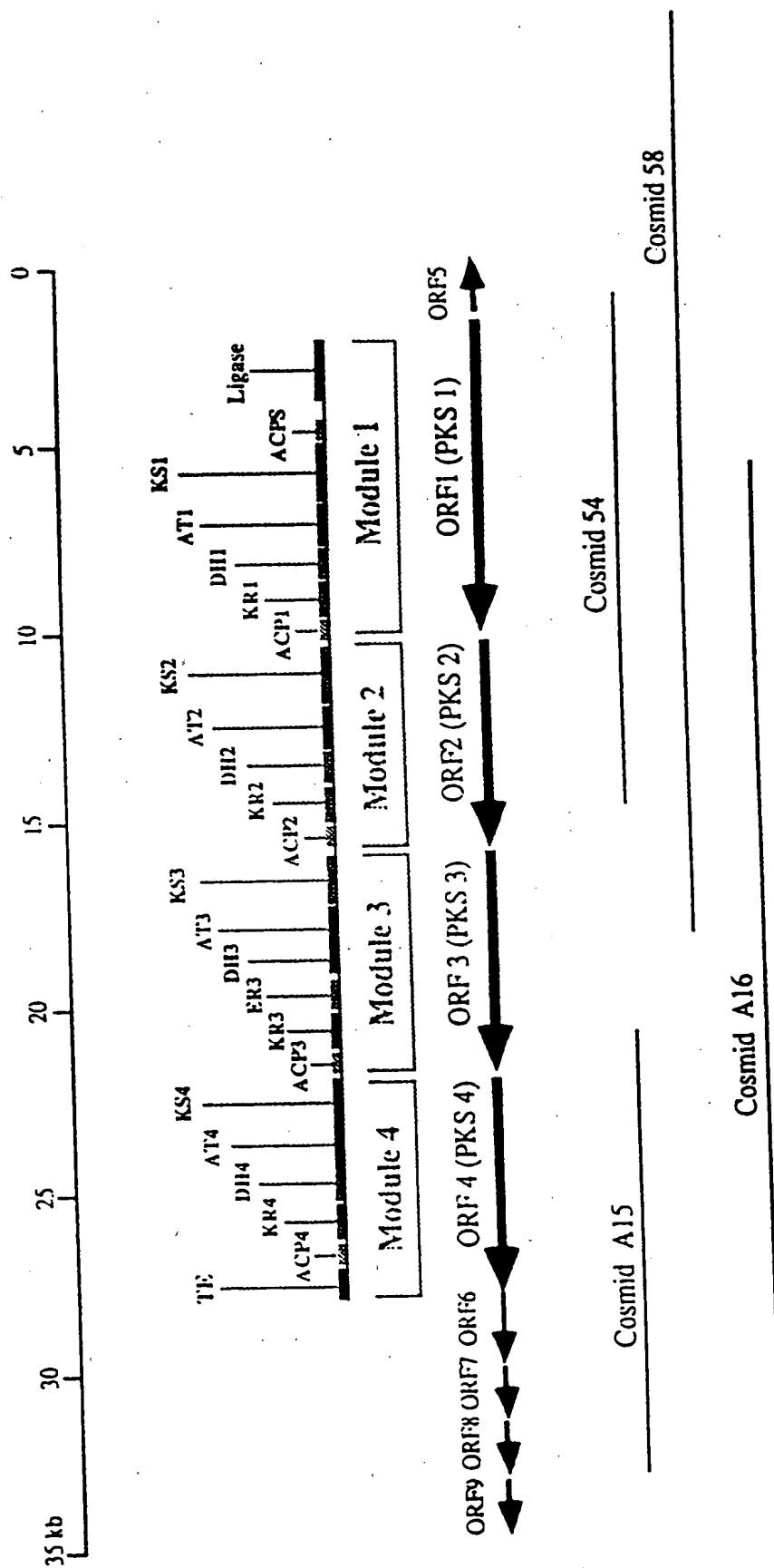


Figure 7

GGGCCGCTGGCGGTGATGTTACCGGACAGGGCTCCCAACGCCCCGGCATGGGACGACAG 60
 G P L A V M F T G Q G S Q R P G M G R Q 20
 TTGTACGAGCACTTCCCCGTCTTCGCCCAGGCACTGGACGAGGTCTTCGCACTCGCCACC 120
 L Y E H F P V F A Q A L D E V F A L A T 40
 CCGGACTACGCGAGGTGATGTTGACCCCGACCAGGCCGAAACACTCCAACGCACCGAC 180
 P G L R E V M F D P D Q A E T L Q R T D 60
 CACGCCCAGATCGCCCTGTTTCGCCTTCGAAACCGCCCTCTACCGACTCTGGGAATCCTGG 240
 H A Q I A L F A F E T A L Y R L W E S W 80
 GGCCTGCGACCCGACATGGTCTGCGGACACTCGGTGCGAGAAATCACCGCAGCCACGTC 300
 G L R P D M V C G H S V G E I T A A H V 100
 TCCGGCACCCCTCACCCCTCCCCGACGCCGTCCACCTCGTCACCACACGCGGCACCCCTCATG 360
 S G T L T L P D A V H L V T T R G T L M 120
 CAAAACCTGCCCCCGGGCGGCCATGCTCGCCGTGCGCCACCGACCCCCACACCCCTCCAA 420
 Q N L P P G G A M L A V A T D P H T L Q 140
 CCCCACCTCGACAACCACACGACACCATCTCCATCGCCGCCATCAACGGCCCCCAGGCC 480
 P H L D N H H D T I S I A A I N G P H A 160
 ACCGTCCCTCTCCGGCGACCGCACCCCTCCACCACATCGCCACCCAACTCAACACCAAA 540
 T V L S G D R T T L H H I A T Q L N T K 180
 ACCAACTGGCTCAACGTCAGCCACGCCTTCCACTCCCCCTCATGCAACCCATCCTCCAA 600
 T N W L N V S H A F H S P L M Q P I L Q 200
 CCCTTCACCACCACCCTCAACACCCTCACCCACCACCCCCACACACACCCCTCATCAGC 660
 P F T T T L N T L T H H P P H T P L I S 220
 ATGCTCACCGCCACACCCACCCACCCCGACACCACCCACTGGACCCAGCACATCACCGCA 720
 M L T A T P T H P D T T H W T Q H I T A 240
 CCGTCCGCTACACCGACACCCCTCCACCACCTCCACCACCACGGCATCACACCTACCTC 780
 P V R Y T D T L H H L H H H G I T T Y L 260
 GAAATCGGCCCCGACACCACCCTCACCGCCCTCGCCCGCACCACCCTCCCCACCACCACC 840
 E I G P D T T L T A L A R T T L P T T T 280
 CACCTCATCCCCACCACCCGCGCAACCACAACGAAGTCCGCAGCACGAACGAGGCGTTG 900
 H L I P T T R R N H N E V R S T N E A L 300
 GGCAGGGTGTTCAGCGTGGGCCACTCGGTGGACTGGCGGGCCCTCACTCCGACCGGGAGG 960
 G R V F S V G H S V D W R A L T P T G R 320
 CGTACCTCCCTGCCGACGTACCCCT 935
 R T S L P T Y P 328

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Figure 8

PCR oligos:

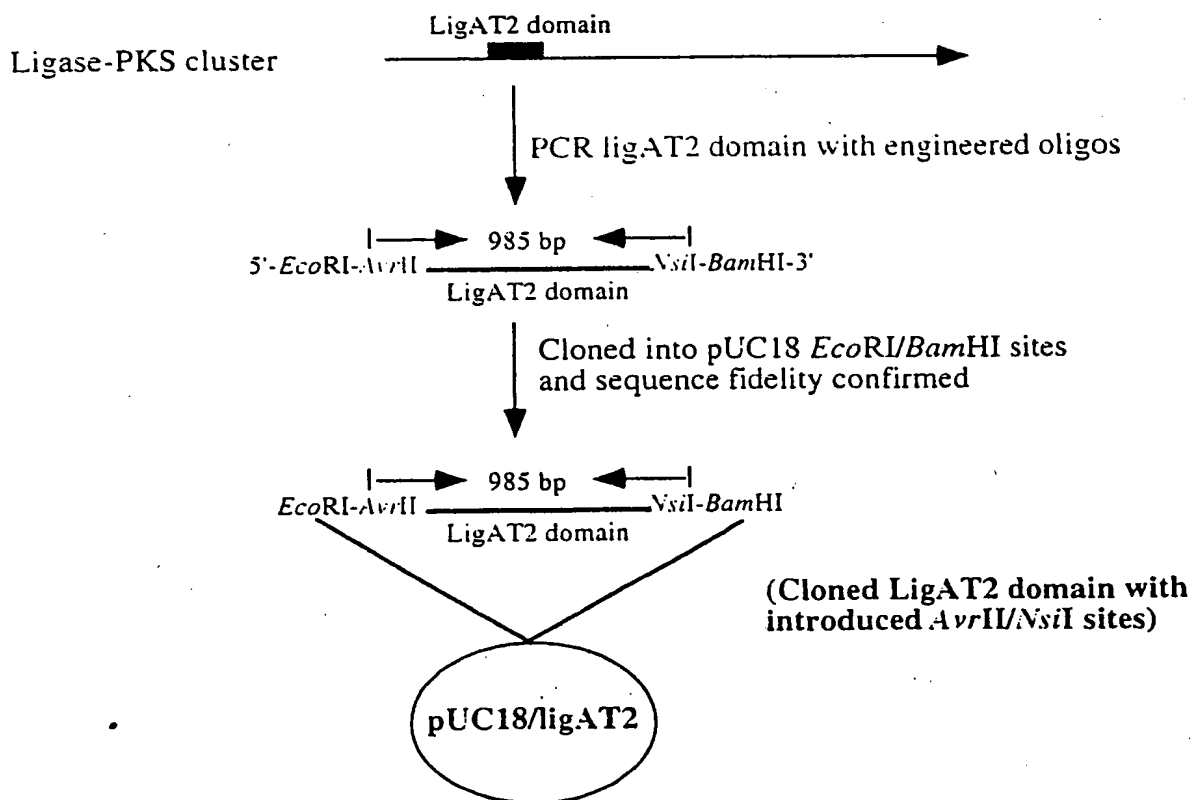
N-terminal Oligo: 5' *EcoRI* Tag-^{AvrII}CCTAGGCTGGCGGTGATGTTCA-3'
GGGCC

Engineered *AvrII* | Homologous region

C-terminal Oligo: 5' *BamHI* Tag-^{NsiI}ATGCATACGTCGGCAGGGAGGTAC-3'
G GG

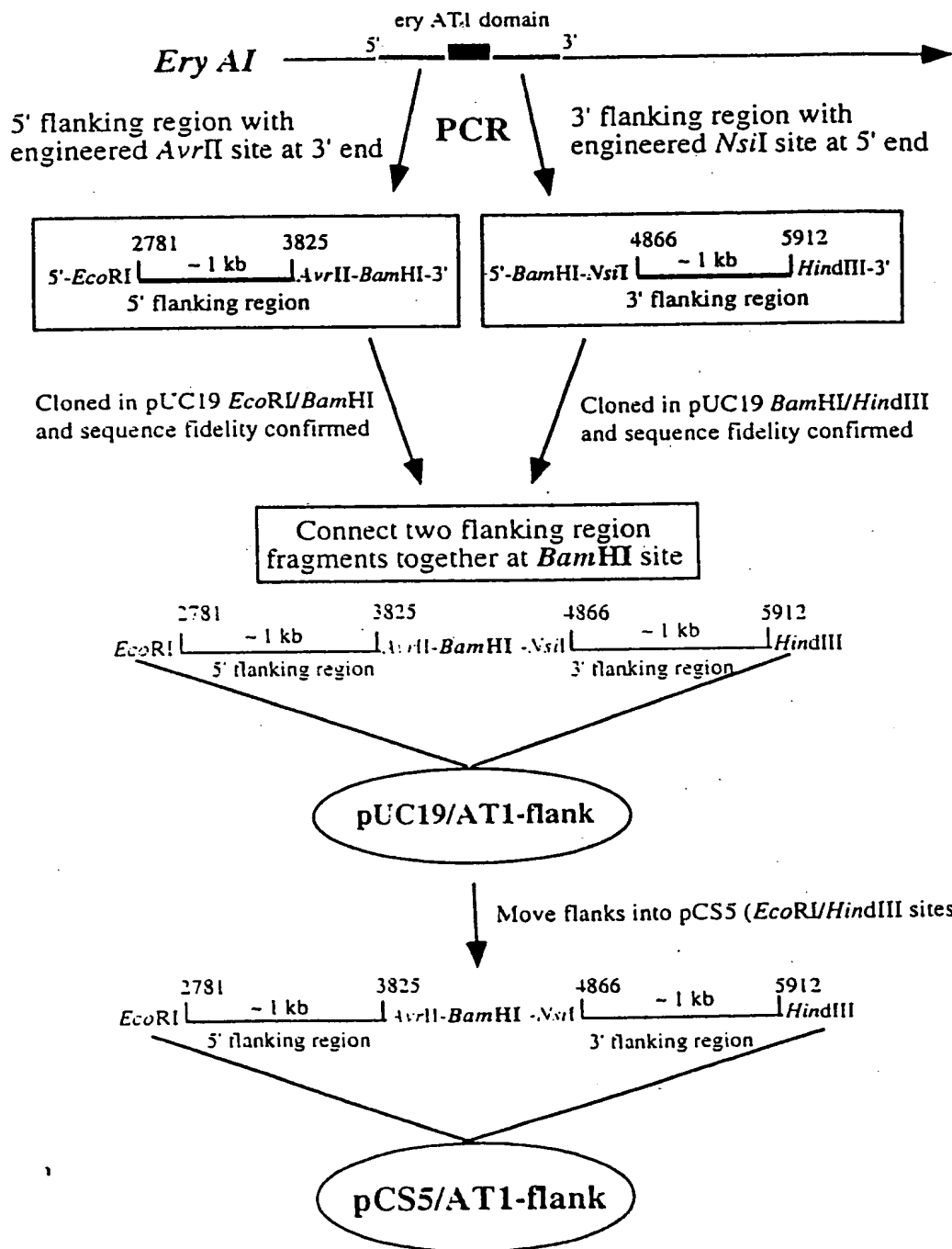
Engineered *NsiI* | Homologous region

PCR cloning:



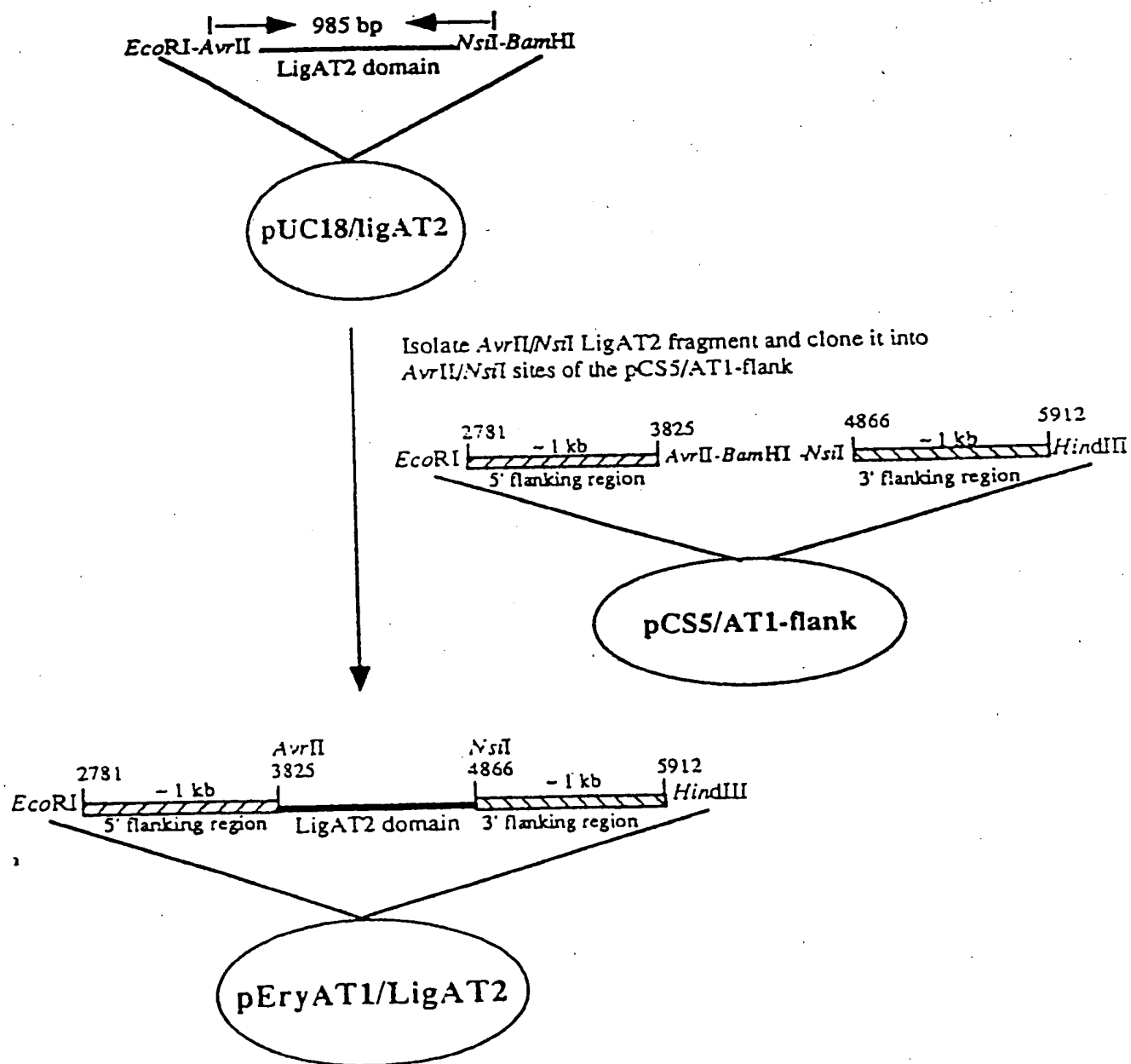
09735056-121100

Figure 9



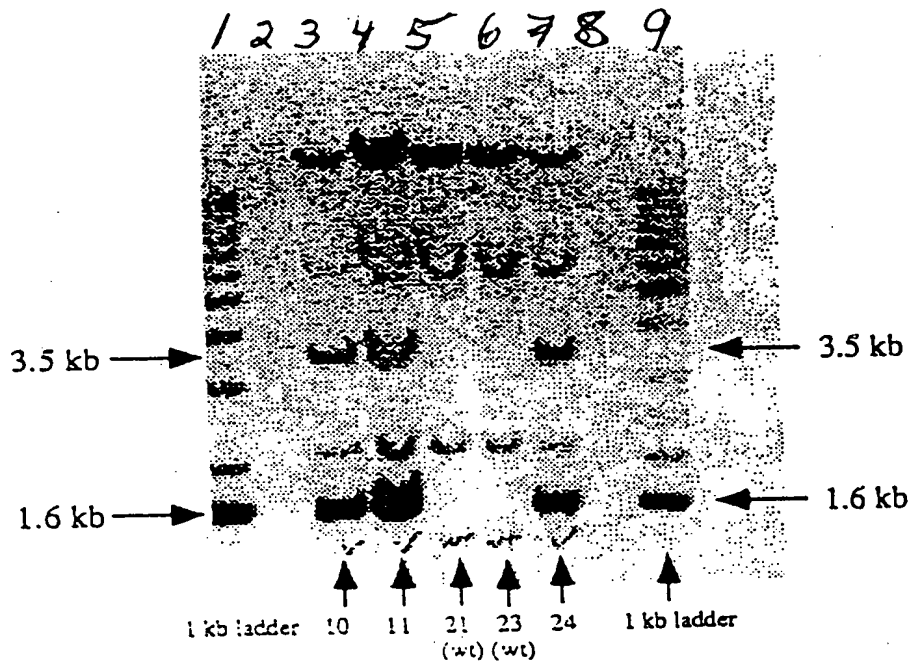
09735055 121100

Figure 10



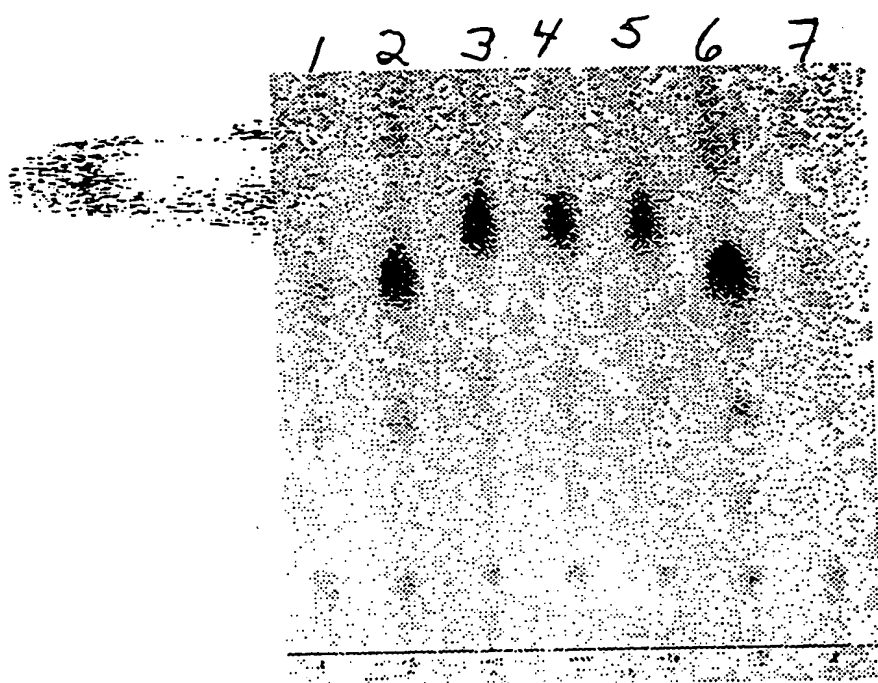
09735056 121100

Figure 11



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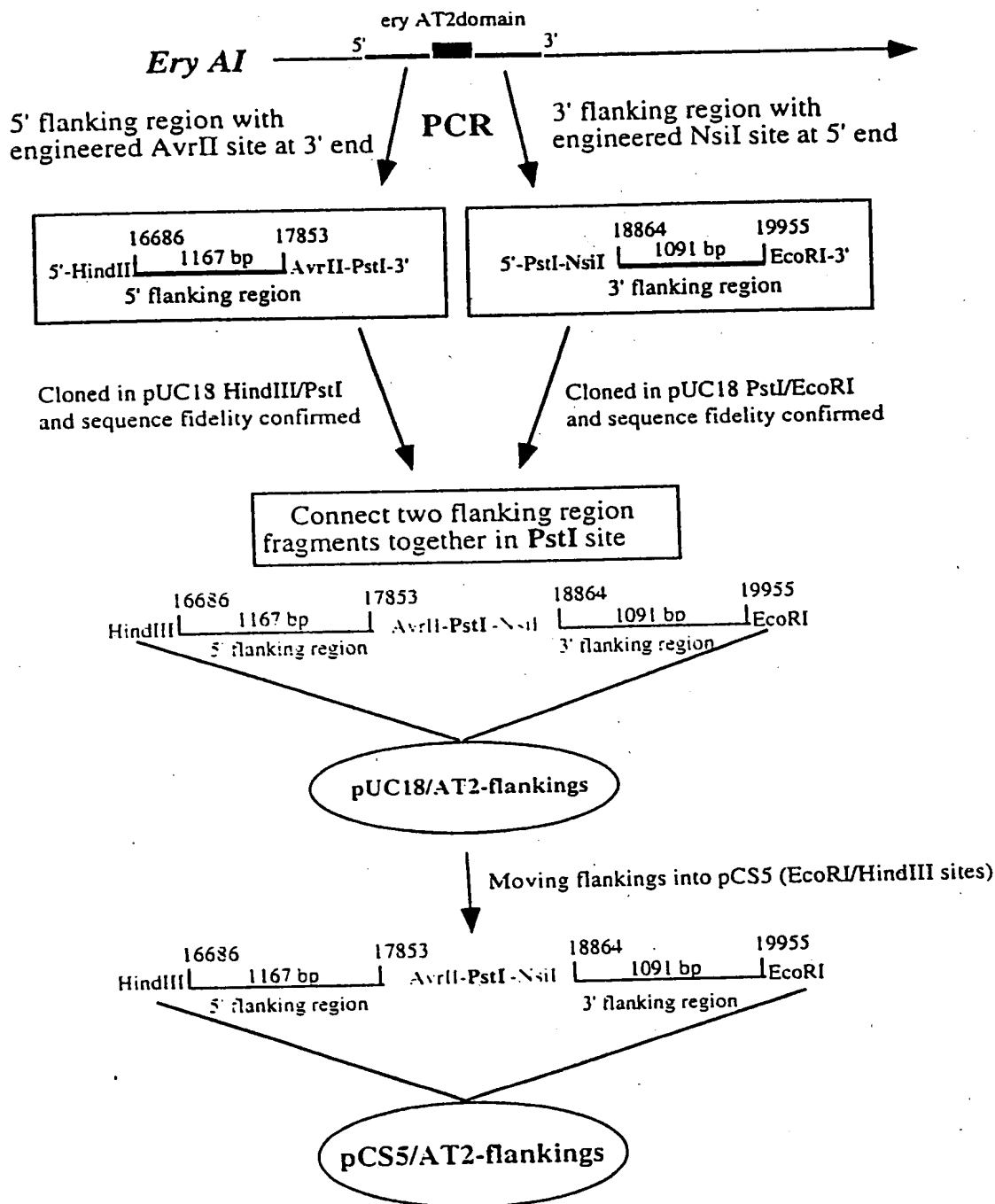
Figure 12



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Figure 13

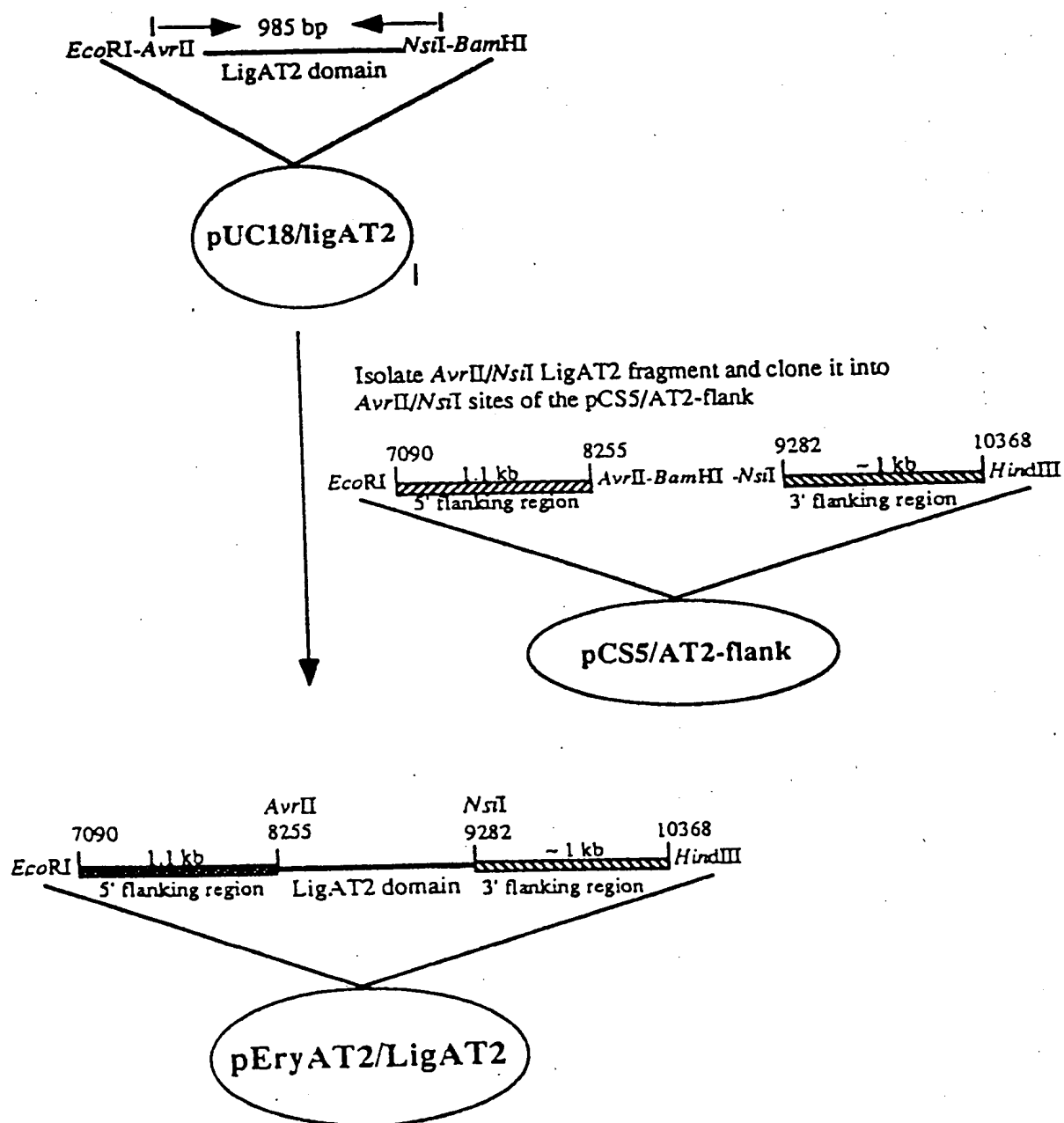
Construction of eryAT2 flanking regions in pCS5



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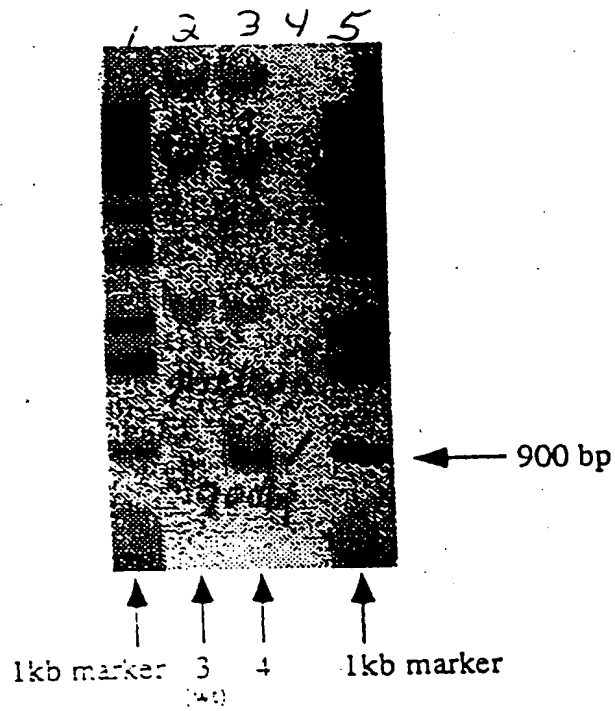
Figure 14

Scheme for Construction of pEryAT2/LigAT2 Integration Plasmid



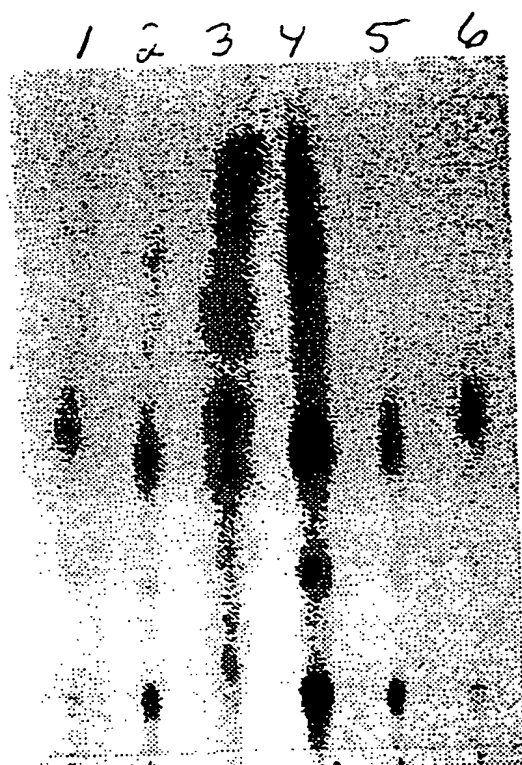
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Figure 15



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Figure 16



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Figure 17

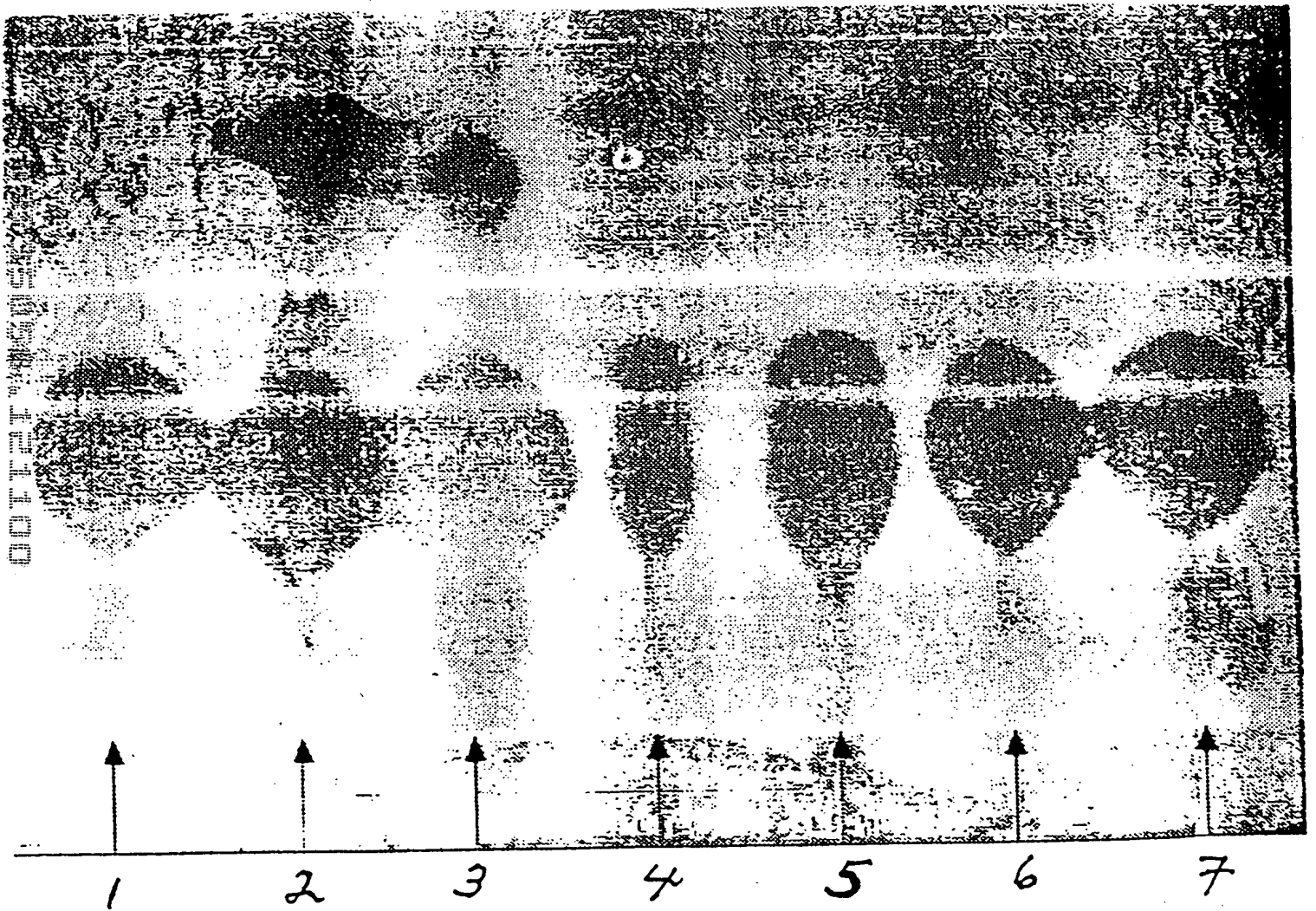


Figure 18

CCTAGGACGGCAGTCCTGCTCACCGGGCAGGGTTCCCAGCGTCAGGGCATGGGGCGCGAA 60
 P R T A V L L T G Q G S Q R Q G M G R E 20
 CTGTACGACCGGTCACCGGTGTTGCGCCGCTCGTTTCGACGCGATCTGCGCTCAACTCGAC 120
 L Y D R S P V F A A S F D A I C A Q L D 40
 GGGCAACTGCCTCGTCCCCCTCAAGGACGTTCTCTTCGCCCCCGAGGGGTGCGAGGACGCC 180
 G Q L P R P L K D V L F A P E G S E D A 60
 GCGCTCATCGACCGTACGGTGTTCACACAGGCGGGCTCTGTTTCGCGGTGGAGACCTCCCTG 240
 A L I D R T V F T Q A A L F A V E T S L 80
 TTCCGGCTGTTTCGAGGCCCCACGGCCTCGTCCCCGACTACCTCATCGGCCACTCCATCGGC 300
 F R L F E A H G L V P D Y L I G H S I G 100
 GAAGTGACCGCGGCCCCACCTGGCCGGGGTCTCGATCTGGCGGACGCGTGCCTGCTGTC 360
 E V T A A H L A G V L D L A D A C V L V 120
 GCCCACCGCGGCGCCTGATGCAGTCGGCCCCGGCGCGGCGCGATGGCCGCGGTCCAG 420
 A H R G R L M Q S A R A G G A M A A V Q 140
 GCGAGCGAGGACGAGGTACGCGAGGCCCTCGCGACCTTCGACGATGCGGTTGCCGTGGCC 480
 A S E D E V R E A L A T F D D A V A V A 160
 GGAGTCAACGGCCCCGAACGCCACCGTCGTCTCCGGCGACGAGGACGCGGTGCGAGCGGCTG 540
 G V N G P N A T V V S G D E D A V E R L 180
 GTCGCGCGCTGGCGCGAGCAGGGCAGGCGGACGAAGCGGCTGCCGGTCAGCCACGCCTTC 600
 V A R W R E Q G R R T K R L P V S H A F 200
 CACTCGCCGCACATGGACGGGATCGTCGACGAGTTCGTACCGCCGTCTCCGGGCTCACC 660
 H S P H M D G I V D E F V T A V S G L T 220
 TTCCGCTCCCCGACGATCCCGGTGCTCTCCAACGTCACCGGGACCCTCGCCACCGTCGAC 720
 F R S P T I P V V S N V T G T L A T V D 240
 CAGCTGACCTCGCCCGCTACTGGGCACGCCACATCCGCGAGGCCGTGCGCTTCGCCGAC 780
 Q L T S P A Y W A R H I R E A V R F A D 260
 GGGGTGCGGTACCTGGAGGGCGAGGGCGTACCGAATGGCTGGAGCTCGGGCCCCGACGGC 840
 G V R Y L E G E G V T E W L E L G P D G 280
 GTTCTCGTCCGCTGGTTCGAGGACTGCCTGGCGAAGGAGGCGGGATCGCTCGCGTCCGCC 900
 V L V A L V E D C L A K E A G S L A S A 300
 CTGCGCAAGGGGGCGAGCGAGCCCCACACCGTGGGCGCGGCCATGGCCCCGCGCGGTGCTG 960
 L R K G A S E P H T V G A A M A R A V L 320
 CGCGGATCCGGCCCCGACTGGGCGGCGGTGTTCCCCGGCGCACGGCGGGTTCGACCTTCGG 1020
 R G S G P D W A A V F P G A R R V D L P 340
 ACGTATGCAT 1030
 T Y A 343

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Figure 19

PCR oligos:

N-terminal Oligo: 5' *Eco*RI Tag-^{AvrII}CCTAGGACGGCAGTCCTGCTCACC-3'
GGCC

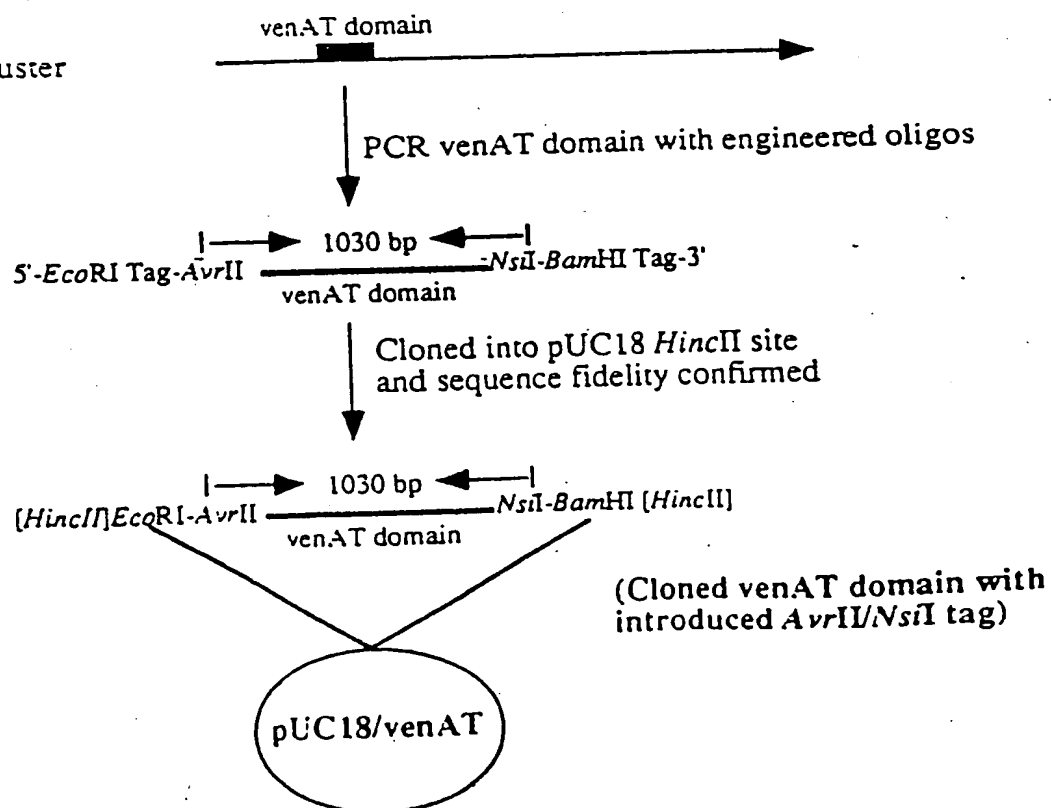
Engineered *Avr*II | Homologous region

C-terminal Oligo: 5' *Bam*HI Tag-^{NsiI}ATGCATACGTCGGAAGGTCGACCCG-3'
C C

Engineered *Nsi*I | Homologous region

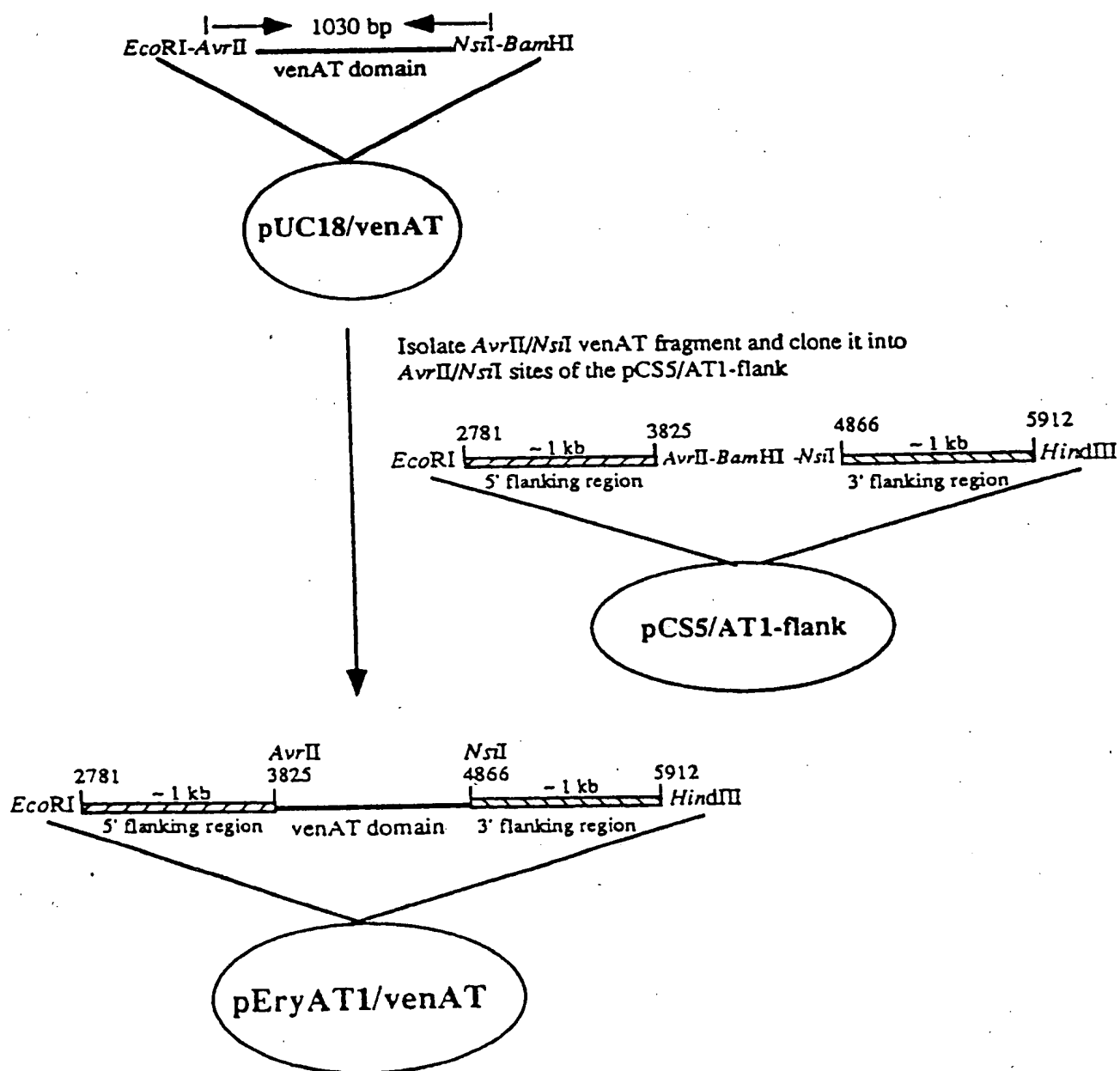
PCR cloning:

Ven-PKS cluster



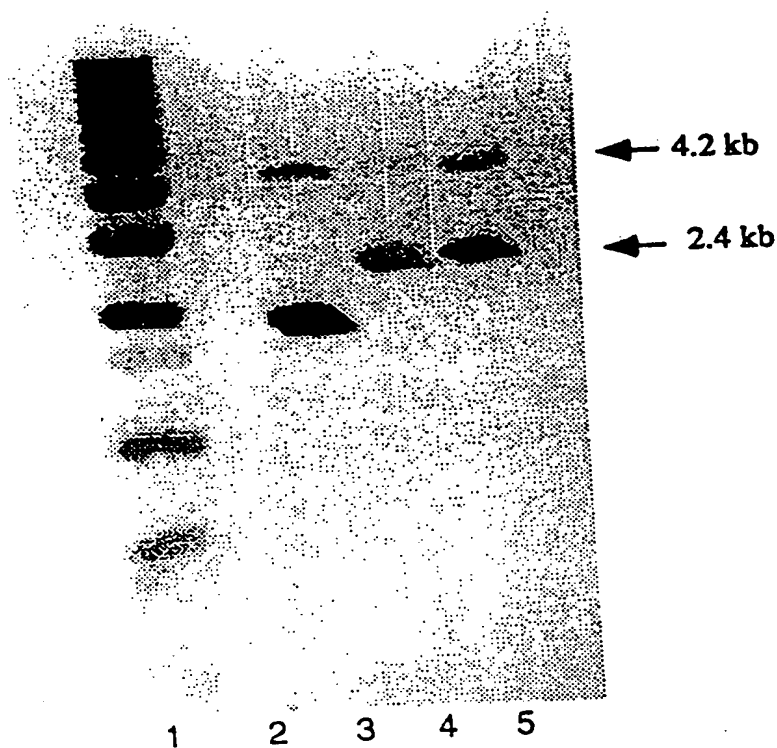
09735056 121100

Figure 20



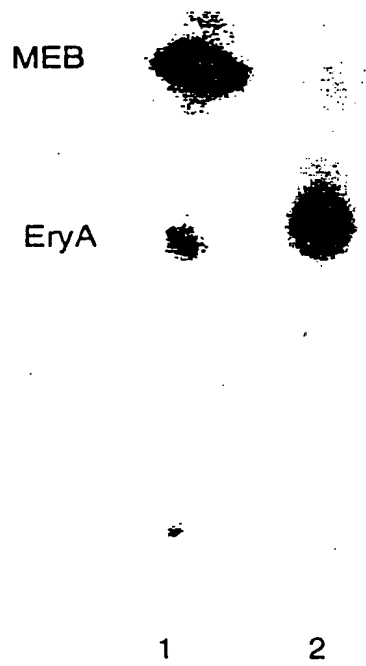
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Figure 21



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Figure 22



09735056 121100

Figure 23

PCR oligos:

N-terminal Oligo: 5' *EcoRI* Tag- $\overbrace{\text{CCTAGGGTTGCCTTCCTGTTTCGAC}}^{\text{AvrII}}\text{-3'}$
 GGC C

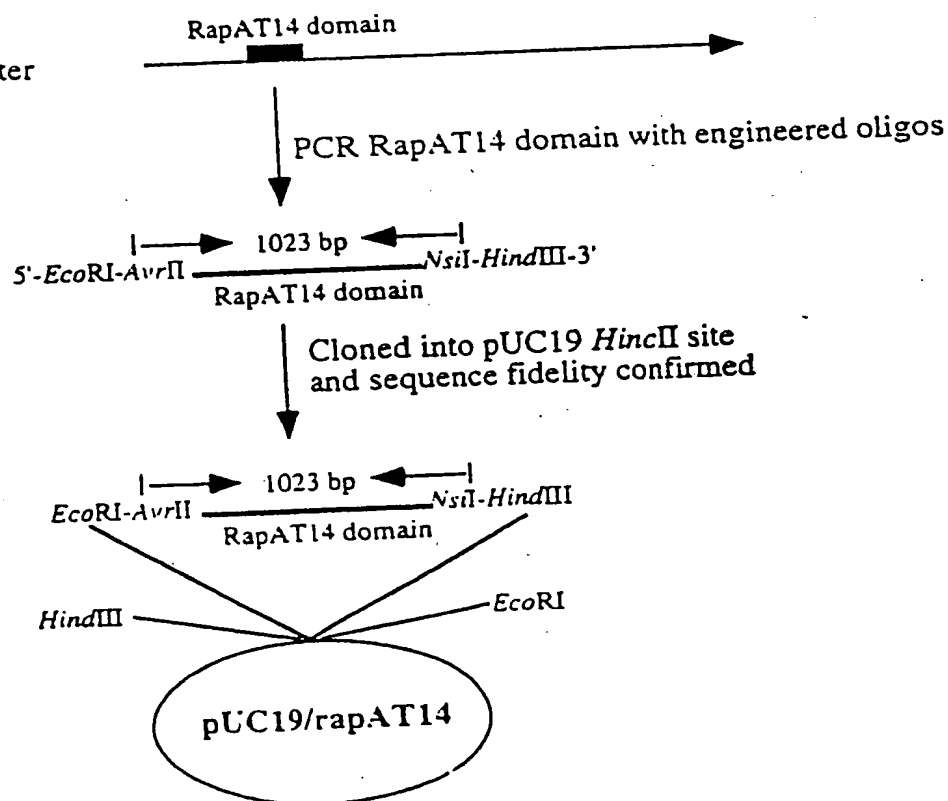
Engineered <i>AvrII</i>	Homologous region
-------------------------	-------------------

C-terminal Oligo: 5' *HindIII* Tag- $\overbrace{\text{ATGCATAGACCGGCAGATCCACCG}}^{\text{NsiI}}\text{-3'}$
 C G

Engineered <i>NsiI</i>	Homologous region
------------------------	-------------------

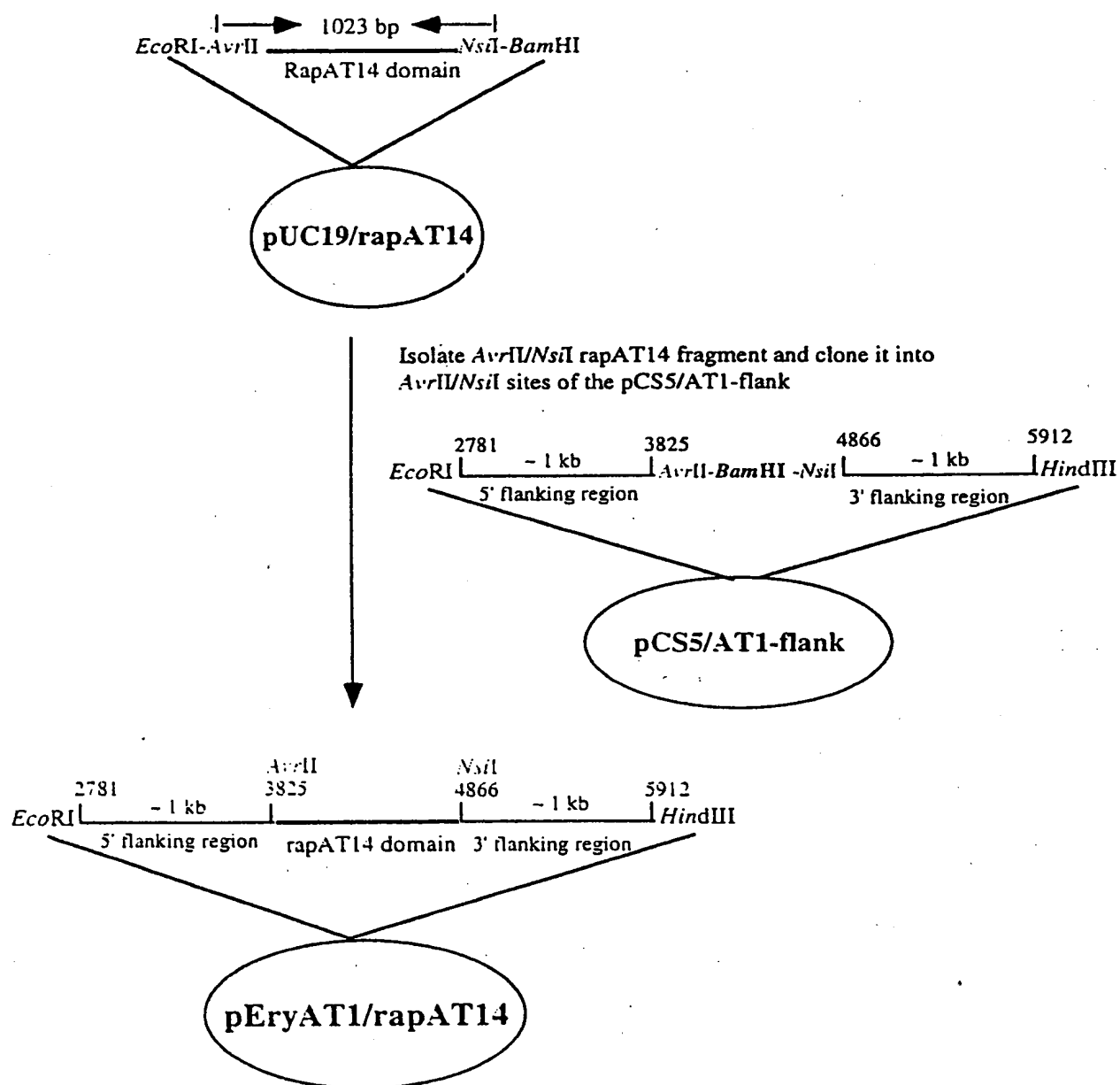
PCR cloning:

Rapamycin cluster



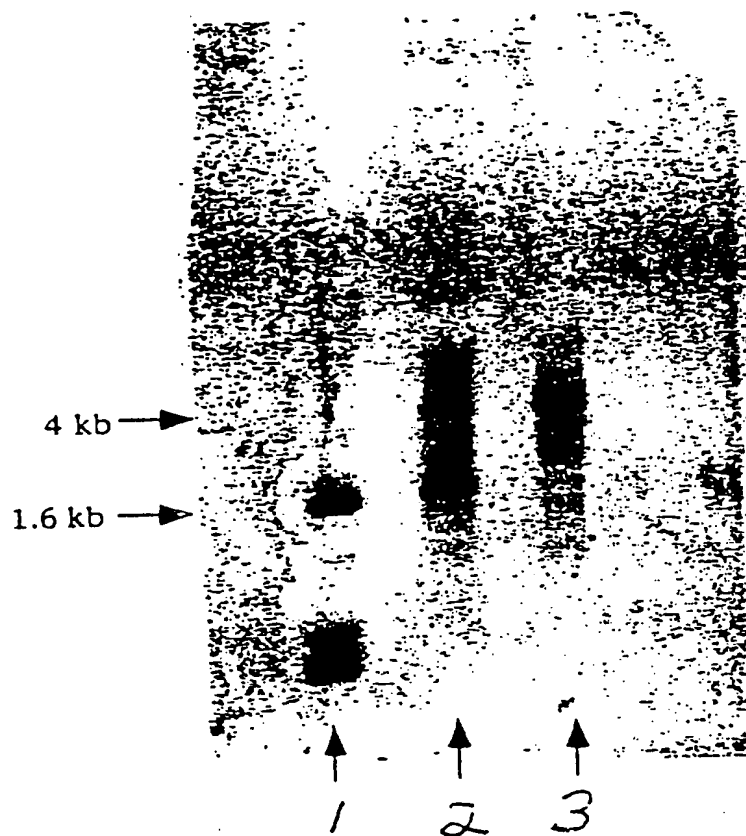
09735056 121100

Figure 24



09735055-121100

Figure 25



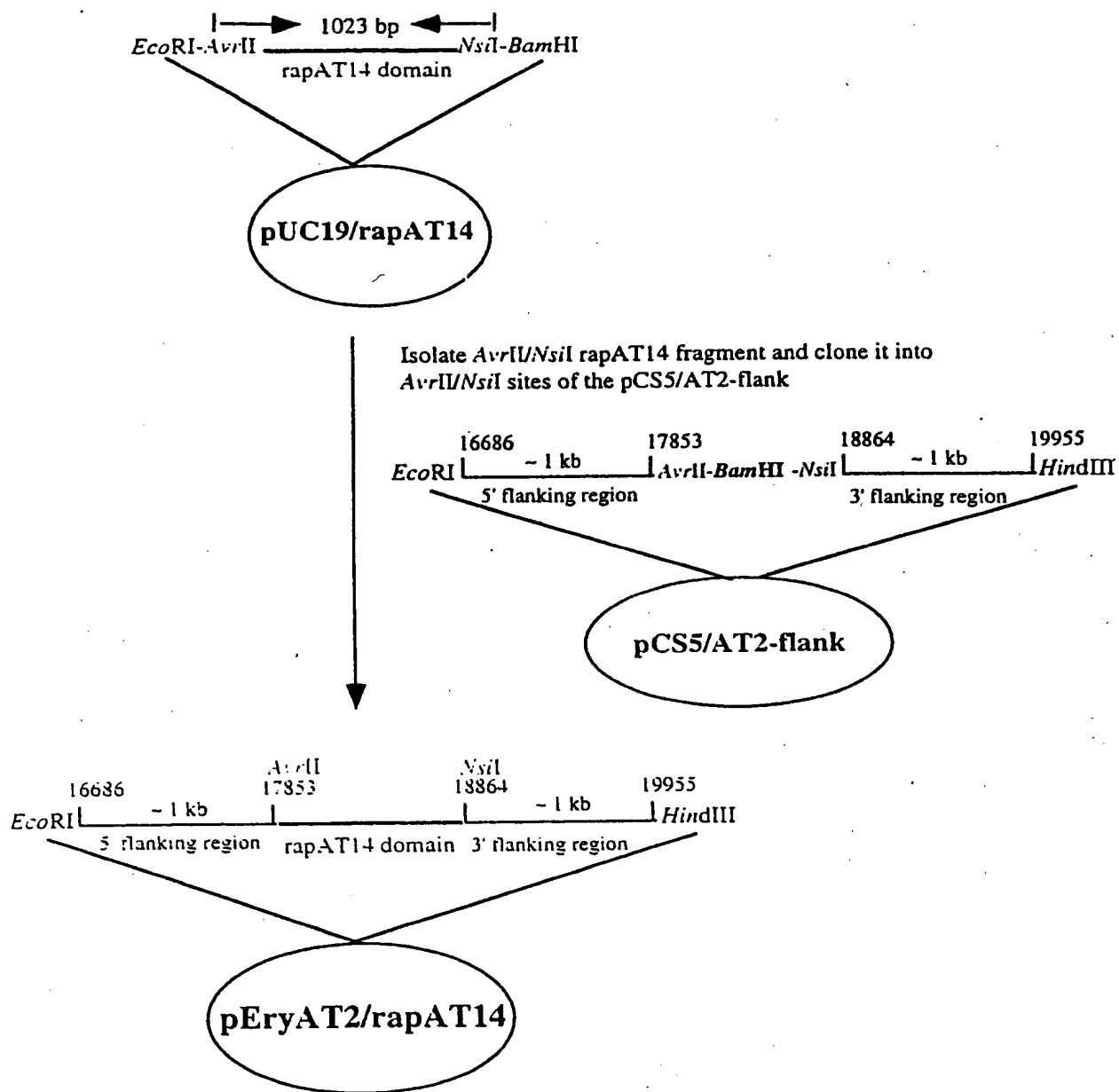
09735056.121100

Figure 26



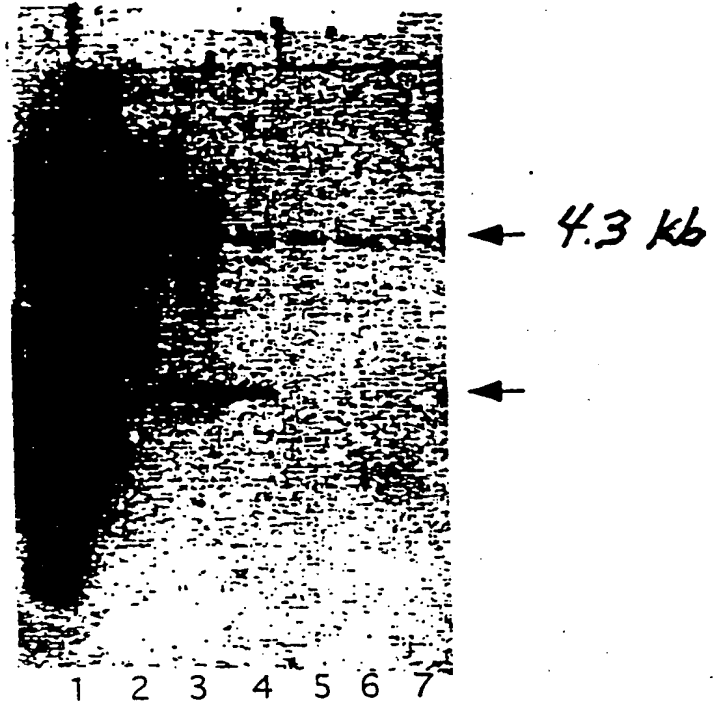
09735056 121100

Figure 27



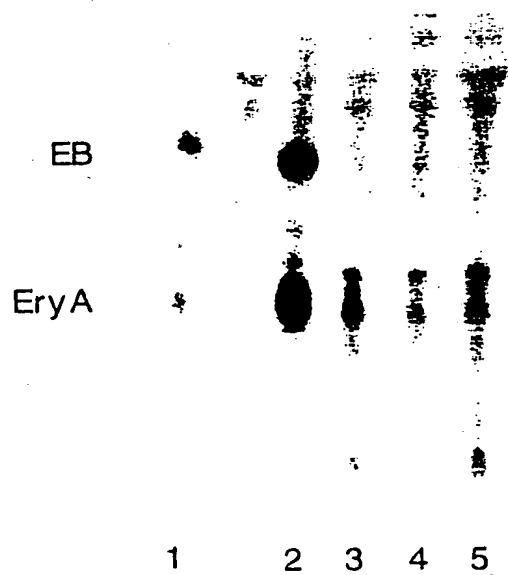
09735056 121100

Figure 28



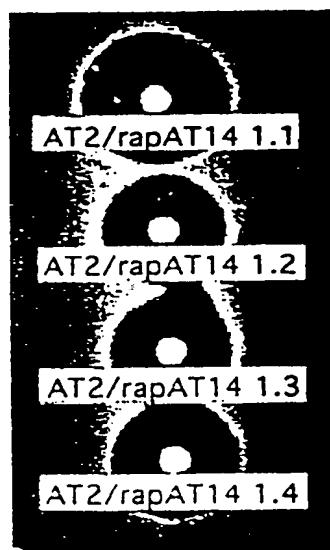
09735056.121100

Figure 29



09735056.121100

Figure 30



09735056 121100

Figure 31

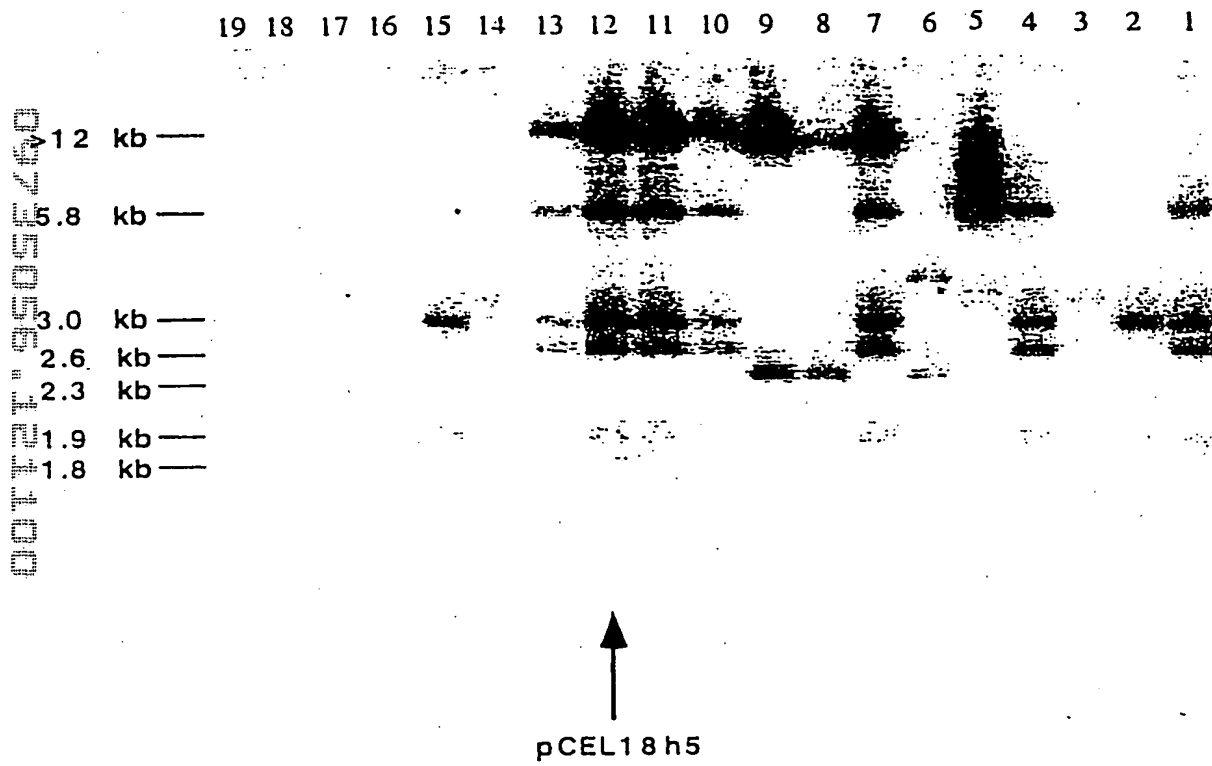


Figure 32

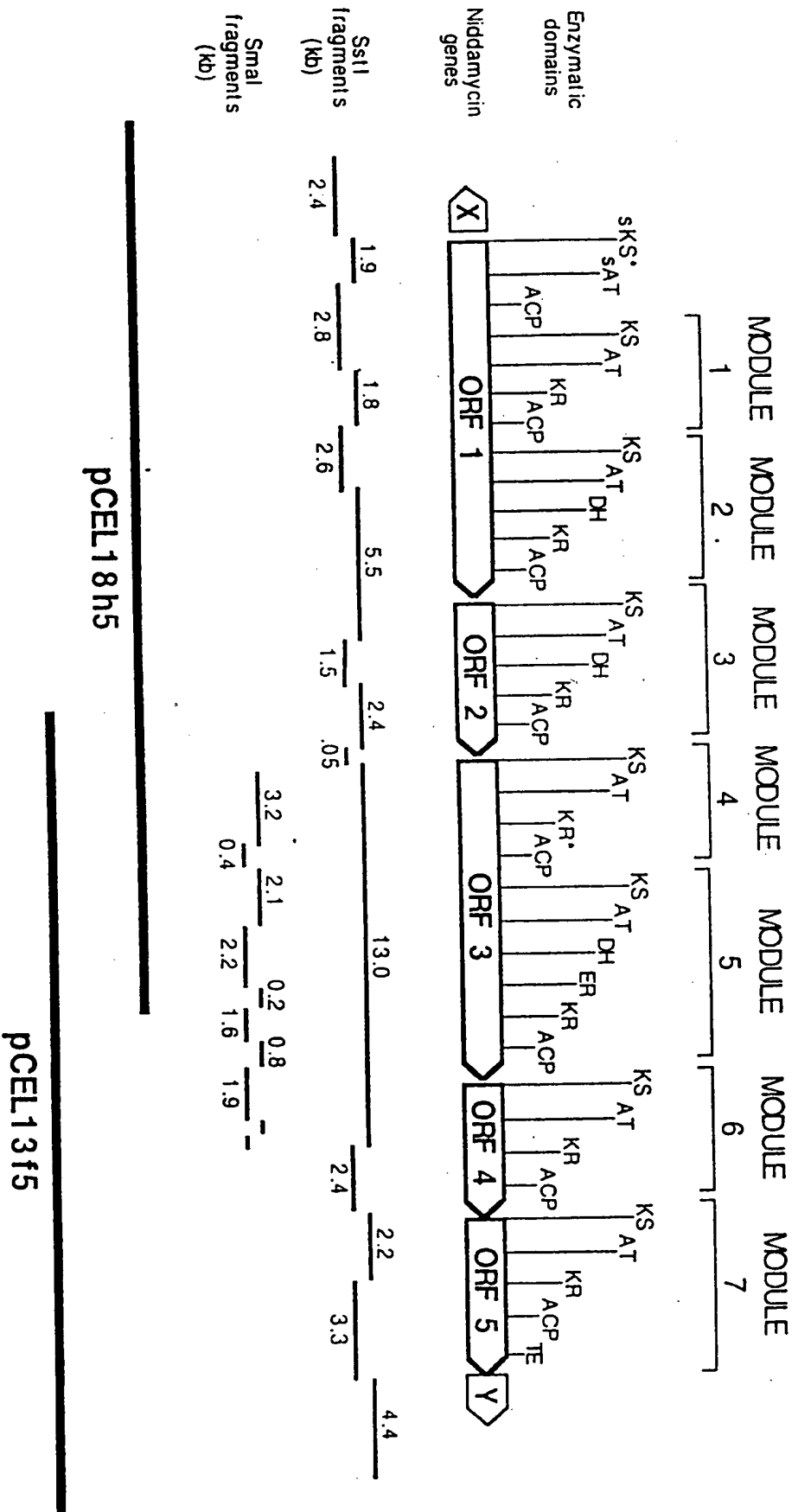
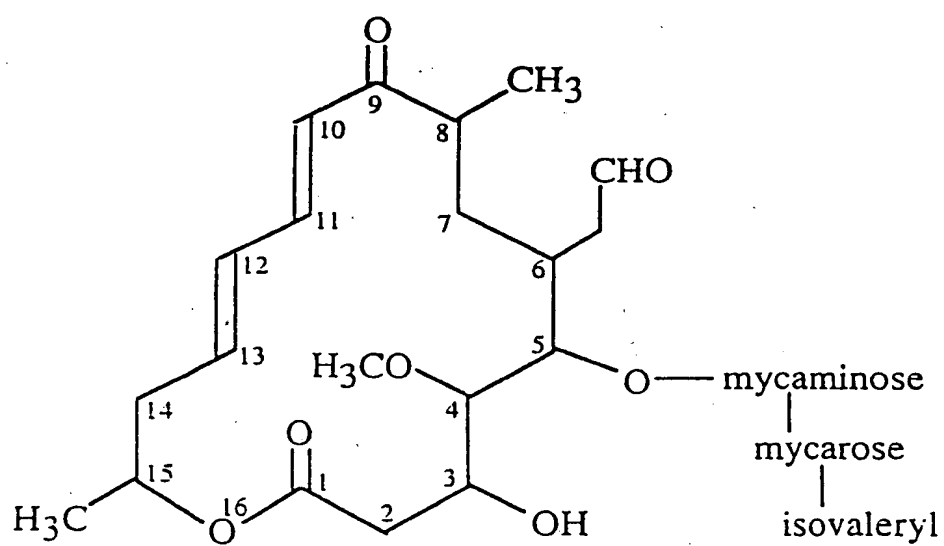


Figure 33



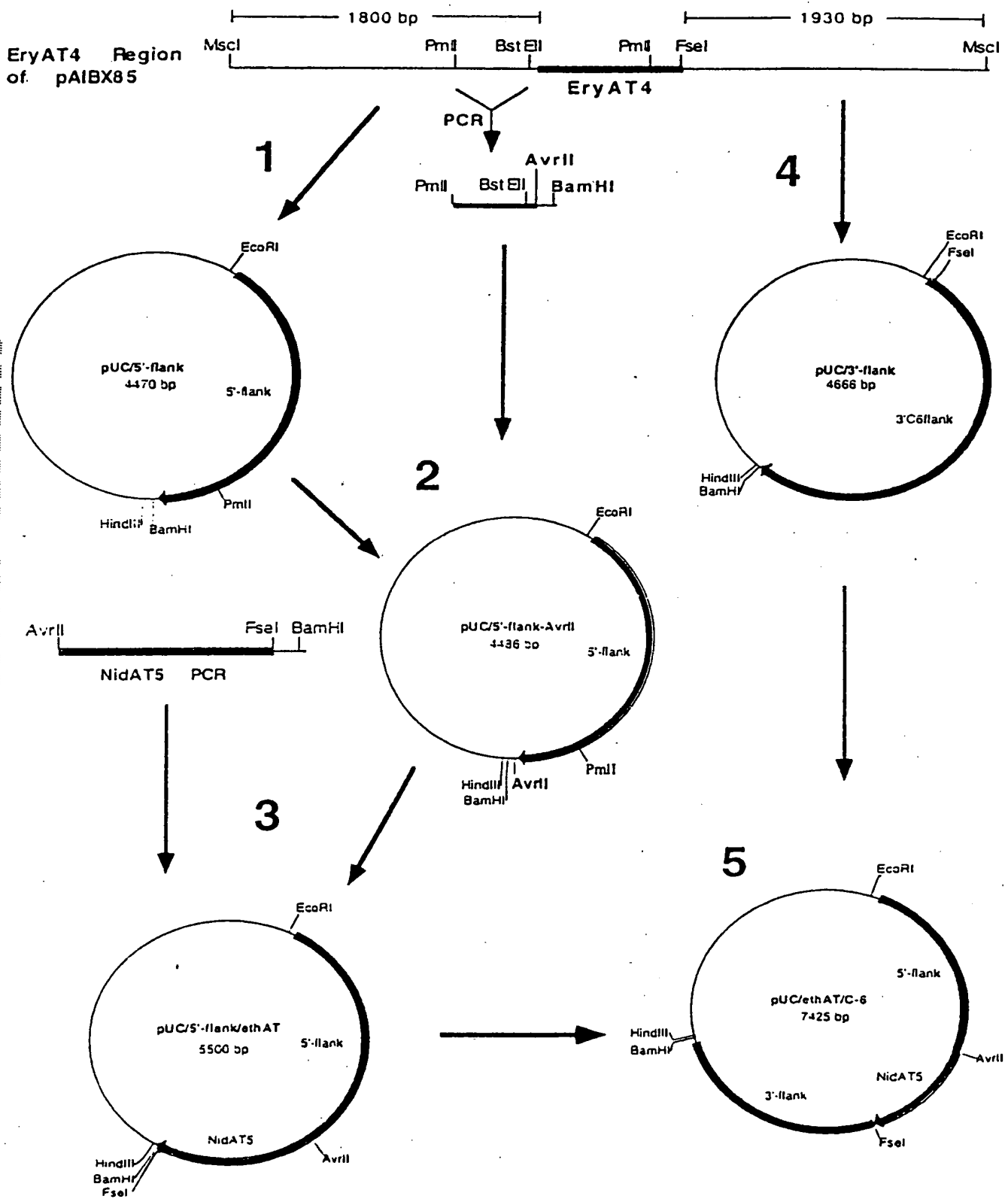
09735056 121100

Figure 34

GCCGACCGTGTCTGTGTTCTGTGTTCCCCGGCCAGGGCTCGCAGTGGGCCCGGAATGGCCGAG 60
 A D R / V F V F P G Q G S Q W A G M A E 20
 GGGCTGCTGGAGCGGTCCGGCGCGTTCGGGAGTGGCGCCGACTCGTGCGACGCCGCGCTG 120
 G L L E R S G A F R S A A D S C D A A L 40
 CGGCCGTACCTCGGCTGGTCGGTGCTGAGCGTGCTGCGCGGGGAACCGGACGCGCCCTCG 180
 R P Y L G W S V L S V L R G E P D A P S 60
 CTCGACCGGGTCGACGTCGTGCAGCCGGTGCTGTTACGATGATGGTCTCGCTCGCGGGC 240
 L D R V D V V Q P V L F T M M V S L A A 80
 GTCTGGCGTGCGCTGGGGGTGGAACCGGCGGCGGTGCTCGGGCACTCGCAGGGTGAGATC 300
 V W R A L G V E P A A V V G H S Q G E I 100
 GCCGCTGCCCATGTCGCCCGGTGCGCTGTCGCTGGACGACTCGGCCCGGATCGTCGCCCTG 360
 A A A H V A G A L S L D D S A R I V A L 120
 CGCAGTCGGCGTGCTCGGACTGGCGGGCAAGGGCGGCATGGTGGCGGTGCCGATGCCG 420
 R S R A W L G L A G K G G M V A V P M P 140
 GCGGAGGAGCTGCGGCCGCGGCTGGTGACGTGGGGGGACCGTCTGGCCGTGCGCCGCCGTC 480
 A E E L R P R L V T W G D R L A V A A V 160
 AACAGCCCCCGTTCTCGCCCGTTCGACGGCGACCCCGAGGGCGCTGGCCGAACCTGGTGGCG 540
 N S P G S C A V A G D P E A L A E L V A 180
 CTGCTGACCGGTGAGGGGGTGACGCCCCGGCCGATCCCCGGCGTTCGACACGGCGGGCCAC 600
 L L T G E G V H A R P I P G V D T A G H 200
 TCGCCCGAGGTGGAGCGCTTGGCGGGCTCATCTGCTGGAGGTGCTGGCCCCGGTGGCCCCC 660
 S P Q V D A L R A H L L E V L A P V A P 220
 CGACCGGGCGACATCCCGTTCTACTCGACGGTGACCGGGCGGGCTGCTGGACGGCACCGAG 720
 R P A D I P F Y S T V T G G L L D G T E 240
 CTGGACGCGACGTAAGTACCGCAACATGCGCGAGCCCGTTCGAGTTCGAGCGGGCCACA 780
 L D A T Y W Y R N M R E P V E F E R A T 260
 CGGGCGCTGATCGCCGACGGGCACGACGTCTTCCTGGAGACGAGCCCGCATCCCATGCTG 840
 R A L I A D G H D V F L E T S P H P M L 280
 GCCGTGCGCGTGGAGCAGACGGTCACCGACGCGCGGCACCGACGCGGGCGGTGCTCGGGACC 900
 A V A L E Q T V T D A G T D A A V L G T 300
 CTGCCCCCGCCGACGGCGGTCTCGCGCGCTGGCCCTGGCCGTCTGCCGCGCCTTCGCG 960
 L R R R H G G P R A L A V C R A F A 320
 CACGGCGTGGAGGTGGAACCCCGAGGCGGTCTTCGGTCCGGCGCGCACGGCCCGTGGAGTTG 1020
 H G V E V D P E A V F G P G A R P V E L 340
 CCCACCTATCCG 1032
 P T Y P 344

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Figure 35



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Figure 26

S A P R K P
TCCGCGCCGCGCAAGCCG
 ↓ ↓ ↓
TCCGCGCC**TAG**AAGCCG
 └────────┘
 AvrII site

09-03-2016

N-Terminal oligo (580-600) 5'-GAGAGAGGAACCAACGCGCACGTGATCGTCGAAGAGGCCACCAGC
 PmlI site
 C-terminal oligo (530-550) 5'-GAGAGAGGATCCGACCTAGGCGCGGAGGTCACCGGCGCGACGGCG
 BamHI site AvrII site

PCR oligos for NidAT5 fragment

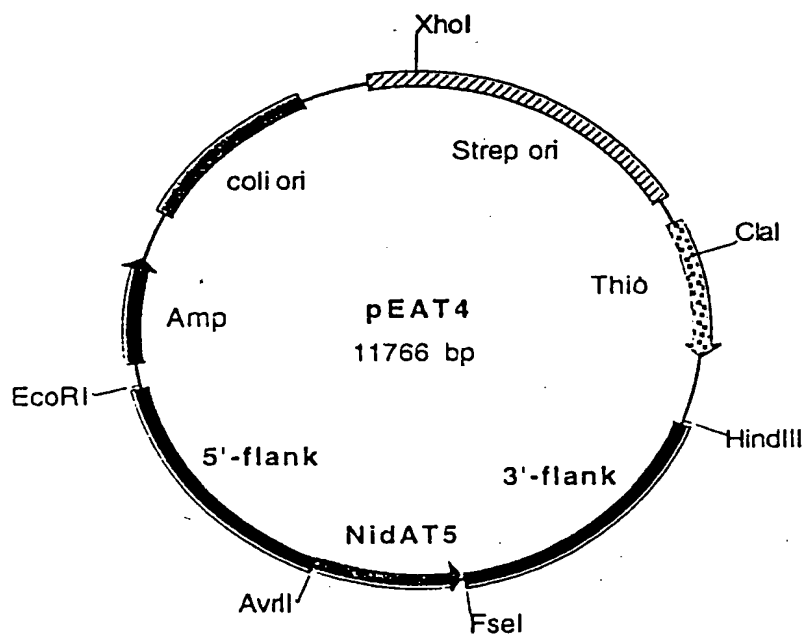
N-Terminal oligo 5'-GAGAGACCTAGGAAGCCGGTGTTCGTGTTCCCCGGCCAGGGCT
(Seq ID no. 23)
AvrII site

Beginning of NidAT5

C-terminal oligo 5'-GAGAGAGGATCCGAGGCGCGCCGTGCGCCCGGACCGAAGACCGCCTC
(Seq ID no. 24)
BamHI site FseI site

3' end of NidAT5

Figure 37



00735056 121100

Figure 38

001121 9505E260

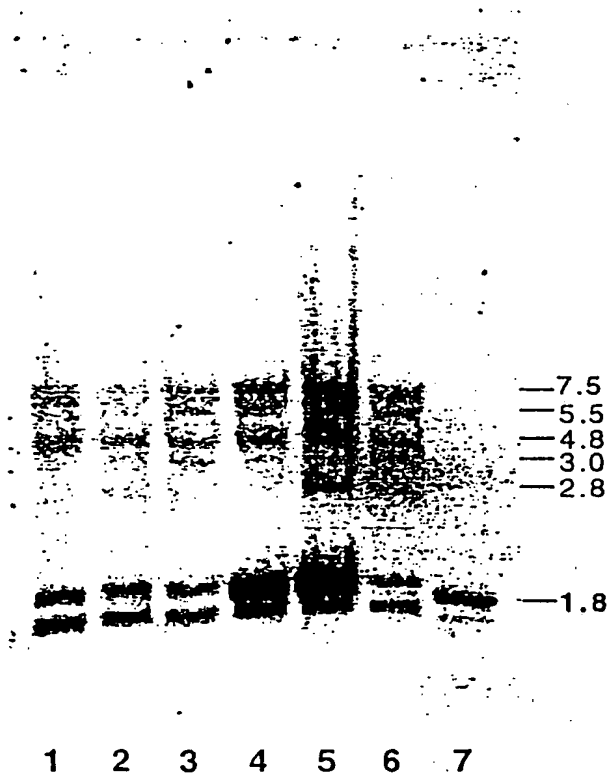
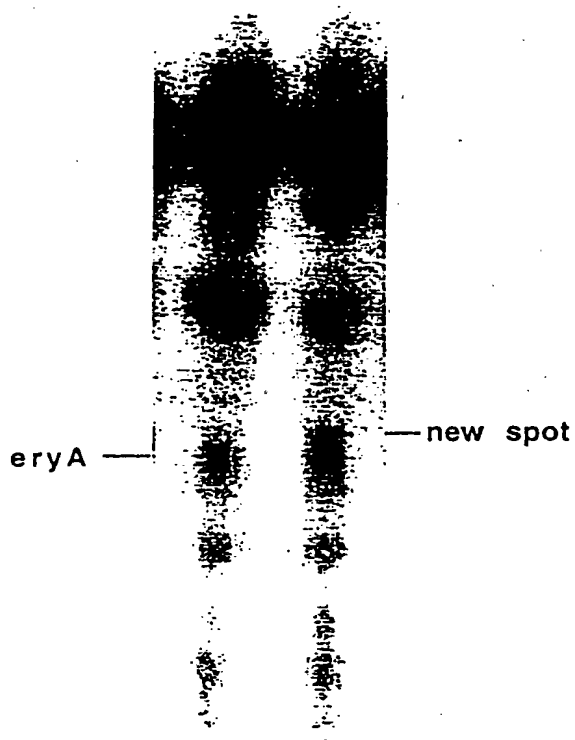


Figure 39



A) SCM only

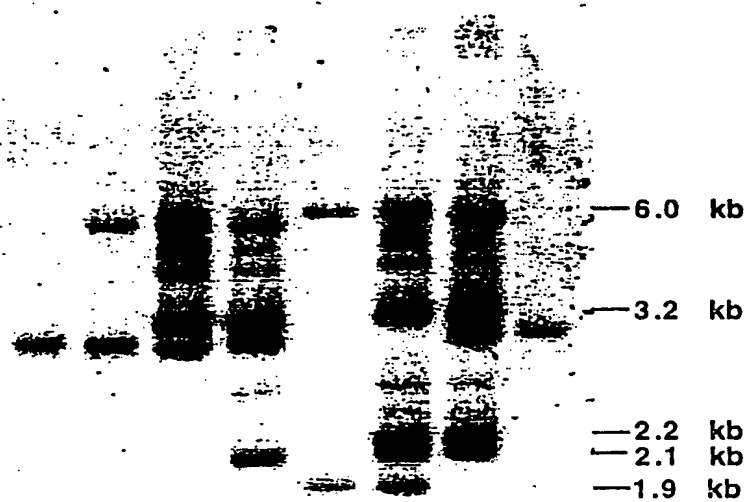
B) SCM + 50mM butyric acid

A

B

09735056-121100

Figure 40



↑
pCEL18h5

↑
pCEL13f5

09735056.121100

Figure 41

CGCGCGCCTGCCTTCGTCTTTCCCGGGCAGGGCGCCAGTGGGCCGGACTGGGAGCGCGG 60
R A P A F V F P G Q G A Q W A G L G A R 20

CTCCTCGCGGACTCCCCGTCTTCCCGGCCAGGGCCGAGGCATGCGCGCGGGCGCTGGAG 120
L L A D S P V F R A R A E A C A R A L E 40

CCTCACCTCGACTGGTCGGTCCTCGACGTGCTGGCCGGCGCCCCGGGCACCCCTCCCATC 180
P H L D W S V L D V L A G A P G T P P I 60

GACCGGGCCGACGTGGTGCAGCCGGTGCTGTTACCACGATGGTCTCGCTGGCCGCCCTC 240
D R A D V V Q P V L F T T M V S L A A L 80

TGGGAGGCCCCACGGGGTGCGGCCGGCCGCGGTCTGGGCCACTCCCAGGGCGAGGTGGCC 300
W E A H G V R P A A V V G H S Q G E V A 100

GCGGCCTGCGTGGCCGGTGCCCTGTCGCTGGACGACGCTGCCCTGGTGATCGCCGGACGC 360
A A C V A G A L S L D D A A L V I A G R 120

AGCAGGCTGTGGGGGCGGCTGGCCGGGAACGGCGGGATGCTCGCGGTGATGGCTCCGGCC 420
S R L W G R L A G N G G M L A V M A P A 140

GAGCGGATCCGTGAGCTGCTCGAACCATGGCGGCAGCGGATTTCCGTGGCGGCGGTCAAT 480
E R I R E L L E P W R Q R I S V A A V N 160

GGCCCCGCTCGGTACCGTCTCCGGTGACGCGCTCGCGCTGGAGGAGTTCGGCGCGCGG 540
G P A S V T V S G D A L A L E E F G A R 180

CTCTCCGCGGAGGGGGTGCTGCGCTGGCCGCTGCCGGGCGTCTCGACTTCGCCGGCCACTCG 600
L S A E G V L R W P L P G V D F A G H S 200

CCGCAGGTGGAGGAGTTCGCGCTGAGCTCCTGGACCTGCTCTCCGGCGTACGGCCGGCT 660
P Q V E E F R A E L L D L L S G V R P A 220

CCTTCGCGGATACCTTTCTTCTCCACCGTGACGGCGGGTCTTTCGCGCGGCGACCACTG 720
P S R I P F F S T V T A G P C G G D Q L 240

GACGGGGCGTACTGGTACCGCAACACGCGGAACCCGTGGAGTTCGACGCCACGGTCCGG 780
D G A Y W Y R N T R E P V E F D A T V R 260

GCGCTGCTGCGTGCGGGCCATCACACGTTTCATCGAGGTGGTCCGCATCCGCTGCTCAAC 840
A L L R A G H H T F I E V G P H P L L N 280

GCCGCGATCGACGAGATCGCAGCGGACGAGGGGGTAGCGGCCACGGCCCTGCATACGCTC 900
A A I D E I A A D E G V A A T A L H T L 300

CAGCGGGGCGCTGGCGGCCTTGACCGCGTGCGCAACGCGGTGGGCGCCGCTTTTCGCGCAC 960
Q R G A G G L D R V R N A V G A A F A H 320

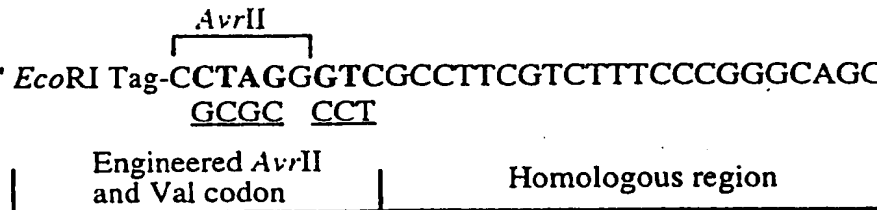
GGTGTCCGGGTGACTGGAACGCCCTGTTTCAGGGCACCGGTGCGCGCAGGGTGCCGCTT 1020
G V R V D W N A L F E G T G A R R V P L 340

CCCTCGTACGCCTTC 1035
P S Y A F 345

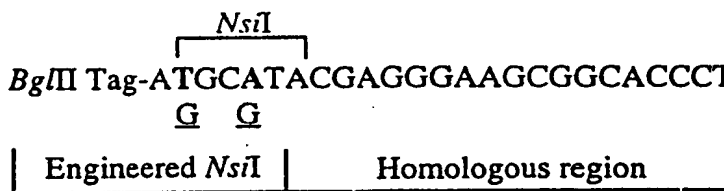
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0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99

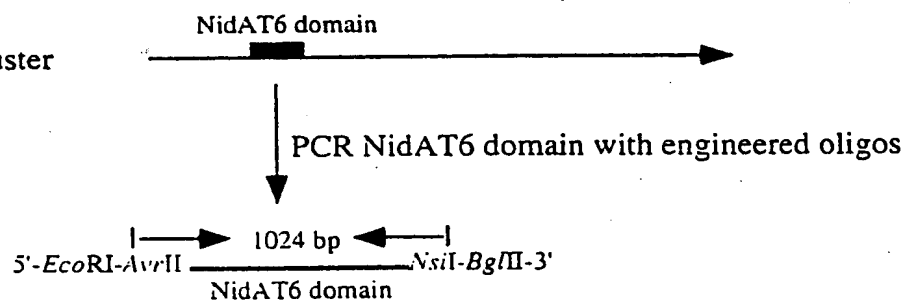
N-terminal Oligo: 5' *Eco*RI Tag-CCTAGG^{AvrII}GTCGCCTTCGTCTTTCCCGGGCAGG-3'
GCGC CCT



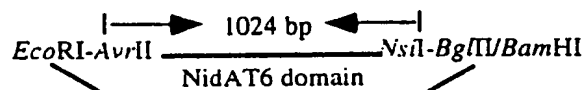
C-terminal Oligo: 5' *Bg*III Tag-ATGCATACGAGGGAAGCGGCACCCTGC-3'



The diagram illustrates the Nidamycin cluster and the NidAT6 domain. A horizontal line represents the Nidamycin cluster, with a black box indicating the NidAT6 domain. An arrow points to the right from the NidAT6 domain. Below the NidAT6 domain, a vertical line connects to the text "PCR NidAT6 domain with engineered oligos".



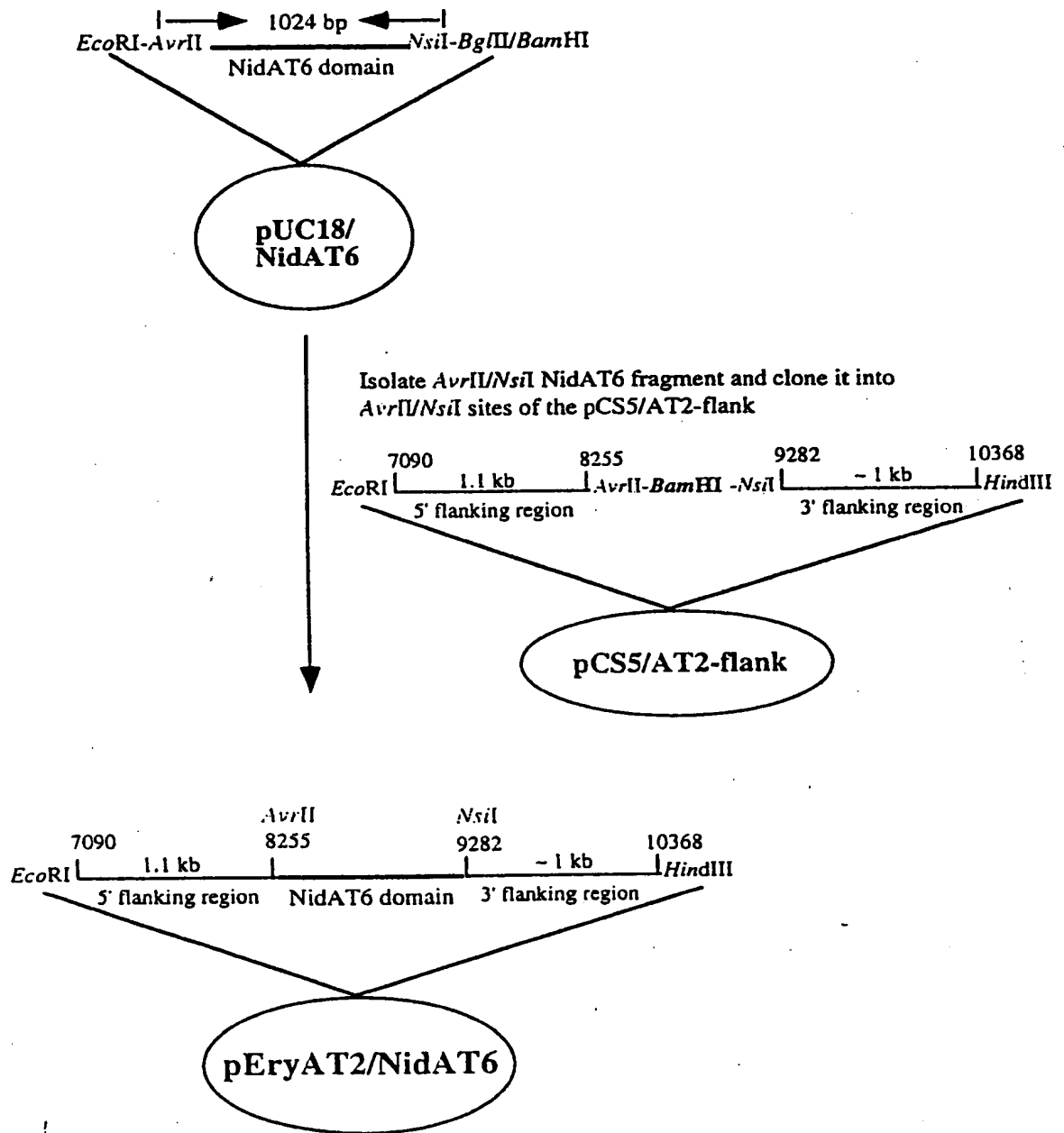
Cloned into pUC18 *EcoRI*-*Bam*HI sites
and sequence fidelity confirmed



(Cloned NidAT6 domain with introduced *AvrII*/*NsiI* sites)



Figure 43



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